Mycelial biology of xylariaceous fungi

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Award date:
1987

Awarding institution:
University of Bath

MYCELIAL BIOLOGY OF XYLARIACEOUS FUNGI

Submitted by Priscilla Rosemary Sharland

for the degree of Ph.D.

of the University of Bath

1987

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ACKNOWLEDGEMENTS

I wish to express my thanks to Dr. A.D.M. Rayner for his continued encouragement, guidance and enthusiasm throughout the course of my research.

I am indebted to all those who supplied me with samples, namely Dr. A.M. Ainsworth, Dr. G. Chamuris, R.C. Dryden, A. Ofong, Dr. A.D.M. Rayner, Dr. N.H.C. Sparks, Dr. A.J.S. Whalley and R. Wolstenholme. Special thanks to D.K. Barrett and A. Ofong for enabling me to accompany them on a field trip to Ainsdale Sand Dunes Nature Reserve, southwest Lancashire.

I am similarly grateful for the useful advice and helpful discussions with Dr. A.M. Ainsworth, Dr. L. Boddy, Dr. J. Kirby, Dr. L.E. Petrini and Dr. A.J.S. Whalley, and for assistance from the technical staff of the Microbiology Department, and to Judy Harbutt for typing this thesis.

Thanks are also due to my parents, friends and colleagues who have helped me in various ways during the past three years.

Finally, I gratefully acknowledge the University of Bath for financial support in the form of a University Research Studentship.

SUMMARY

Various aspects of the mycelial biology of some wood-decaying xylariaceous species were investigated. These included the genetically-based variation in their mycelial characteristics, vegetative mycelial transitions between morphologically different forms, their intraspecific and interspecific recognition reactions and occurrence of temporary heterokaryosis and the distribution of individual genotypes in natural populations.

Each of the species studied had distinctive mycelial characteristics and some exhibited considerable developmental plasticity, producing zones or sectors of different mycelial types. This ability to alternate between morphologically and possibly functionally distinct developmental modes may be important during colonization.

Studies of intraspecific interactions showed that demarcation zones between different strains displayed a spectrum of recognition phenomena resulting in varying degrees of non-self rejection and acceptance, which appeared to be affected by the degree of genetic difference between self and non-self. Non-self acceptance involved the formation of a temporary heterokaryon in the demarcation zone, the form of which resembled some of those associated with mating in outcrossing Basidiomycotina.

Based on the presence of somatic incompatibility and variability between ascospore progeny from the same perithecium most of the populations investigated appeared to be sexually outcrossing. Hypoxylon multiforme, "Hypoxylon purpureum" and Rosellinia desmazieresii seemed predominantly to be sexually non-outcrossing; their ascospore progeny were culturally similar and somatically compatible.

Analysis of the spatial structure of natural populations demonstrated that in the standing tree species existed either as a limited number of genotypes occupying considerable domain, or as numerous genotypes with small domains. Colonization appears to have been via latent invasion from bases established endogenously in the xylem, or exogenously in the bark respectively.

Species could be arranged in a combative hierarchy according to their ability to defend or replace other species. Daldinia concentrica and Hypoxylon nummularium were the most and least combative species respectively.

Ecological strategies, life cycle and host selectivity were discussed.

NOTES ON NOMENCLATURE

1. Fungi

Taxonomic terms throughout this thesis accord with "Ainsworth and Bisby's Dictionary of the Fungi" (Hawksworth, Sutton and Ainsworth, 1983). All organisms referred to in the text have been described previously according to the authorities listed below, with the exception of a variant form collected during the study which was superficially similar to *Hypoxylon rubiginosum*, but distinct from the latter in colour, life cycle, mycelial characteristics and habitat relationships. For convenience this form has been referred to as "*Hypoxylon purpureum*", but it must be stressed that this is not a legitimate taxon.

Acrostaphylus Arnaud ex Subramanian

Anthostomella Sacc.

Armillaria (Fr.) Staude

Armillaria bulbosa (Barla) Romagn.

Armillaria mellea (Vahl.) Quél.

Ascobolus crenulatus Karst

Ascobolus immersus Pers.

Aspergillus nidulans (Eidam) Wint.

Biscogniauxia O. Kuntze

Camillea Fr.

Ceratocystis ulmi Buism.

Chondrostereum purpureum (Fr.) Pouz.

Cochliobolus heterostrophus Drechsler

Coprinus sterquilinus Fr.

Coprinus heptemerus Lange and Smith

Coriolus versicolor L. ex Fr.

Cryphonectria parasitica Murr. Barr.

Cryptostroma corticale (Ell. and Ev.) Greg. and Waller

Daldinia Ces. and de Not.

Daldinia concentrica (Bolt. ex Fr.) Ces. and de Not.

Daldinia vernicosa (Schw. ex Fr.) Ces. and de Not.

Diaporthe phaseolorum (Cook and Ellis) Sacc.

Diatrype Fr.

Endothia parasitica (Murr.) And.

Entonaema Möller

Fusarium oxysporum Schlecht.

Gelasinospora tetrasperma Dowding

Geniculosporium Chesters and Greenhalgh

Hadrotrichum Funckel

Hypocopra (Fr.) Kick X

Hypoxylon Bull. ex Fr.

Hypoxylon argillaceum Berk.

Hypoxylon atropunctatum (Schw.: Fr.) Cke.

Hypoxylon atropurpureum (Fr.:Fr.) Fr. var brevistipitatum var. nov.

Hypoxylon chestersii Rogers and Whalley

Hypoxylon cohaerens Pers. ex Fr.

Hypoxylon cohaerens Pers. ex Fr. var. microsporum Rogers and Candoussau

Hypoxylon confluens (Tode. Fr.) Westd.

Hypoxylon deustum (Hoffm. et Fr.)

Hypoxylon diathrauston Rehm.

Hypoxylon fragiforme (Pers.: Fr.) Kick X

Hypoxylon fuscum Pers.: Fr.

Hypoxylon gwyneddii Whalley, Edwards and Francis

Hypoxylon haematostroma Mont.

Hypoxylon hydnicolum (Schw.) Sacc.

Hypoxylon mammatum (Wahl.) Miller

Hypoxylon mediterraneum (de Not.) Miller

Hypoxylon multiforme Fr.

Hypoxylon notatum Berk. and Curt.

Hypoxylon nummularium Bull.: Fr.

Hypoxylon pruinatum (Klotz.) Cke.

Hypoxylon rubiginosum Pers.: Fr.

Hypoxylon serpens (Pers. ex Fr.) Kick X

Hypoxylon terricola Mill.

Hypoxylon tinctor (Berk.) Cke.

Hypoxylon truncatum (Schw. ex Fr.) Mill.

Hypoxylon udum (Pers.) Fr.

Hypoxylon venustuissimum Pouzar

Hymenochaete corrugata (Fr.: Fr.) Lèv.

Hypholoma fasciculare (Huds. ex Fr.) Kummer

Inonotus dryadeus (Pers. ex Fr.) Murr.

Inonotus hispidus (Bull. ex Fr.) Karst.

Irpex Fr.

Kretzschmaria Fr.

Kretzschmaria clavus (Fr.) Sacc.

Lenzites betulina (Linnemann ex Fries) Fries

Lenzites trabea Pers. ex Fr.

Lopadostroma turgidum (Pers. ex Fr.) Traverso

Monilinia fructicola (Wint.) Honey

Nectria (Fr.) Fr.

Nectria haematococca (Berk. et Br.)

Neurospora crassa Shear et Dodge

Nodulisporium Preuss.

Nummularia Tul.

Nummularia broomeiana (Berk. and Curt.) J.H. Miller

Nummularia discreta (Schw.) Tul.

Ophiostoma ulmi (Buisman) Nannf.

Penicillium claviforme Bainier

Peniophora gigantea (Fr.) Massee

Penzigia frustulosa (Berk. and Curt.)

Periconiella Sacch.

Phaeolus schweinitzii (Fries) Patouillard

Phallus impudicus Pers.

Phanerochaete velutina (Fries) Karsten

Phellinus tremulae (Bond.) Bond. and Borisov

Phylacia Léveillé

Piptoporus betulinus (Bull. ex Fr.) Karst.

Podosordaria Ell. and Holway

Podosordaria leporina (Ellis and Everh.) Dennis

Podospora anserina (Cesati) Niessel

Poronia Willd. ex Fr.

Poronia oedipus (Mont.) Mont.

Poronia pileiformis (Berk.) Fr.

Poronia punctata (L.ex Fr.) Fr.

Psathyrella hydrophila (Bull. ex Mérat) Maire

Pyricularia oryzae Cav. Genovesi

Rhinocladiella Nannf.

Rhopalostroma D. Hawksw.

Rosellinia De Notaris

Rosellinia desmazieresii (Berk. and Br.) Sacch.

Rosellinia herpotrichioides Hepting and Davidson

Rosellinia limoniispora Ell. and Ev.

Rosellinia mammiformis (Persoon ex Fries) Cesati and de Notaris

Rosellinia necatrix (Hart.) Berl.

Schizophyllum commune Fries

Serpula lacrimans (Wulfen: Fr.) Schröter

Sistotrema brinkmannii (Bresadola) Eriksson

Sporothrix Hektoen and Perkins

Stereum hirsutum (Willd.: Fr.) S.F. Gray

Stereum gausapatum (Fr.) Fr.

Stereum sanguinolentum (Alb. and Schw.) Fr.

Thamnomyces Ehrenb.

Thanatephorus cucumeris (Frank) Donk (Rhizoctonia solani Kuhn)

Trichoderma Pers.

Tricholomopsis platyphylla (Pers. ex Fr.) Sing.

Typhula trifolii Rostr.

Ustulina Tul.

Ustulina deusta Tul.

Ustulina vulgaris Tul.

Ustulina zonata Lév.

Virgariella S. Hughes

Wawelia Namyslowski

Wawelia octospora Minter and Webster

Xylaria Hill ex Grev.

Xylaria carpophila Pers: Fr.

Xylaria fioriana Sacc.

Xylaria hypoxylon (L. ex Fr.) Grev.

Xylaria johorensis Morgan Jones and Lim

Xylaria longipes Nitschke

Xylaria magnoliae J.D. Rogers

Xylaria mali Fromme

Xylaria nigripes (Fr.) Dennis

Xylaria oxyacanthae Tul.

Xylaria polymorpha (Pers.) Grev.

Xylaria schweinitzii Berk. et Curt.

2. Plants

Acer L.

Acer pseudoplatanus L.

Alnus Miller

Alnus glutinosa L. Gaertn

Alnus tenuifolia Nutt.

Aloe ferox Mill.

Betula L.

Betula pendula Roth.

Calluna vulgaris (L.) Hull

Carpinus L.

Cedrus atlantica (Endl.) Carr.

Corylus L.

Corylus avellana L.

Crataegus L.

Erica cinerea L.

Fagus L.

Fagus sylvatica L.

Fraxinus L.

Fraxinus excelsior L.

Hedera helix L.

Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell.-Arg.

Macadamia integrifolia Maiden and Beche

Magnolia L.

Mahonia nervosa Nutt.

Malus Miller

Narcissus L.

Picea A. Dietr.

Picea sitchensis (Bong.) Carr.

Pinus nigra var. maritima (Ait.) Melville

Platanus L.

Populus L.

Populus alba L.

Populus grandidentata Michx.

Populus tremula L.

Populus tremuloides Michx.

Pseudotsuga menziesii Mirh. Franco

Pyrus L.

Quercus L.

Quercus rubra L.

Quercus suber L.

Salix L.

Salix repens L.

Sorbus L.

Tilia vulgaris Hayne

Tsuga (Antoine) Carrière

Ulex europaeus L.

Ulex gallii Planch.

Ulmus L.

Vaccinium myrtillus L.

3. **Animals**

Saperda Fabricius

Saperda concolor J. Leconte

Saperda inornata Say

CHAPTER 1

INTRODUCTION

1.1 Background

i. The Xylariaceae

(a) Morphology and taxonomy

The Xylariaceae comprise one of the best recognized groups of Ascomycotina consisting of many genera and species widely distributed throughout the world (Miller, 1949). Twenty-eight genera were included in the family by Eriksson and Hawksworth (1985) who also indicated a further three which might belong there. The Xylariaceae are placed in the largest class of Ascomycotina, the Pyrenomycetes, on the basis that the asci are unitunicate and are borne in parallel series in flask-shaped perithecia, which ultimately open by an apical pore or ostiole. With iodine the apical apparatus of the asci usually gives a blue reaction, although the reaction may vary according to the method used (Nannfeldt, 1976). This character places the family in the order Sphaeriales. The Xylariaceae are not believed to be closely related to any extant families, though they may be distantly related to several, including the Diatrypaceae and the Sordariaceae (Rogers, 1979a).

For most members of the Xylariaceae it is difficult to obtain fertile stromata in culture (Kenerley and Rogers, 1976) although some species can complete their life cycles on artificial media. These species include Poronia oedipus (Jong and Rogers, 1969),

Penzigia frustulosa (Jong and Rogers, 1970), Podosordaria leporina (Rogers, 1973), Daldinia vernicosa, Hypoxylon argillaceum (Whalley, 1979), Hypoxylon gwyneddii (Whalley, Edwards and Francis, 1983) and Hypoxylon atropurpureum var. brevistipitatum (Petrini and Rogers, 1986). Certain strains of Hypoxylon serpens regularly produce perithecia in culture. However, each of these has only been isolated once, their stromata have not been found under natural conditions and their status in nature is unclear (Petrini and Rogers, 1986). They are H. serpens "Barron's strain";isolated from soil (Kenerley and Rogers, 1976), H. serpens "Carroll's strain", an endophyte isolated from Pinus nigra (Jensen, 1981) and H. serpens "Petrini isolate", an endophyte isolated from Mahonia nervosa (Petrini and Rogers, 1986). In contrast to the limited recorded number of species which produce a teleomorph in culture, several investigators have successfully obtained mature stromata with perithecia and asci, by inoculating living trees with ascospores. For example this was accomplished with Hypoxylon atropunctatum (Thompson, 1956), Ustulina deusta, Kretzschmaria clavus and Xylaria schweinitzii (Ko, 1979).

The development of Hypoxylon species in wood and the production of fertile, mature perithecia was reviewed by Miller (1961). The events described probably equally apply to other genera in the Xylariaceae and so will be briefly outlined here. Following ascospore germination hyphae ramify between the bark and cortex and very fine hyphae permeate into the cortex and xylem, the limits of the mycelium being marked in the wood by narrow black lines (these

will be discussed later). Eventually the hyphal network in the bark swells so that the bark tissue is ruptured and the hyphae coalesce to form a stroma, composed of an ectostroma which forms the outer coat of the mature stroma, and an underlying entostroma. Once the ectostroma is exposed, its surface becomes covered in a mat of one-celled conidia and simultaneously, directly below, in the periphery of the entostroma, the perithecial initials form, suggesting that the conidia function primarily as spermatia (Miller, 1961).

Hypoxylon mammatum(synonymous with Hypoxylon pruinatum) differs from other Hypoxylon species as its conidia and perithecial initials do not form on the stroma. The conidia develop either on conidial-pillars, superficially resembling coremia, that rupture the periderm, or on conidiophores arising directly from the subperidermal subiculum. Beneath these conidial regions the perithecial initials are produced "naked" within "nests" of vegetative hyphae and are surrounded by stromata only after being ensheathed in envelopes. Trichogynes are formed in this species and although their function is not determined, it is hypothesized that conidia, serving as spermatia, fuse with them and effect fertilization of ascogonia (Rogers and Berbee, 1964).

The perithecial initials originate as hyphal coils and in the centre of each coil a large diameter, septate hypha - the archicarp - develops, the compartments of which give rise to ascogenous hyphae and in turn croziers and asci. The archicarp is surrounded by fine hyphae that become the perithecial wall which is drawn out

at the apex to become the ostiole (perithecial neck). Cylindrical stalked, unitunicate asci, interspersed with filiform, branched paraphyses, line the base and walls of the mature perithecium, each ascus usually containing eight semi-globose to elliptical one-celled ascospores which orientate uniseriately or diagonally uniseriately and vary from light brown to opaque. The ascospores, which in some species have a longitudinal germ slit through which they germinate, are released through a pore in the apically thickened region of the ascus and are ejected in black masses outside the ostiole (Miller, 1961).

Conidia of all Hypoxylon species, and indeed all species in the Xylariaceae so far investigated, are produced singly and originate blastically from conidiogenous areas of conidiophores (Stiers, Rogers and Russell, 1973), in a sympodial sequence of development (Jong and Rogers, 1972). The origin of the conidium wall has been examined in Poronia punctata (Stiers, Rogers and Russell, 1973), Xylaria johorensis (Morgan-Jones and Hashmi, 1973) and Poronia pileiformis (Paden, 1978) and is holoblastic, that is all the wall layers of the conidiogenous cell are involved in the formation of the conidium wall. Xylariaceous conidia are hyaline to light brown, ovoid to nearly globose (Petrini and Petrini, 1985) and characteristically have a flattened basal scar, often marked by thickened wall material, indicating the area of attachment to the conidiogenous cell. The morphology of the secession scar is distinctive for a given species (Rogers, 1979a).

Although the patterns of development described above serve as a general model for the group, there are numerous variations - for example in stromal form and texture, ostiole, ascospore measurements and conidiophore morphology - which are used in delimitation of different genera and species. Three of these features, stromal form, ostiole type and conidiophore morphology will now be discussed briefly.

Within the family, resupinate, semi-globose and more or less upright or branched forms of stromata occur (Figure 1.1) and the number of perithecia contained by a stroma may vary from one, for example in some species of Rosellinia, to many, for example in some Hypoxylon species. With regard to ostioles there are three types - papillate, annulate and umbilicate (Martin, 1967a). The majority of the Xylariaceae have papillate ostioles in which the ostiolar neck penetrates the ectostroma (Martin, 1967a) and forms a distinctive protruberance beyond the stromal surface (Whalley, 1977). The perithecial neck in umbilicate ostioles does not penetrate the ectostroma (Miller, 1961), so that the necks are flush with the stromal surface and appear as small pores (Whalley, 1977). Annulate ostioles are formed by the sloughing off of a circular plaque of ectostroma around the ostiole, leaving part of the entostromal crust exposed (Martin, 1967a).

The conidiophore morphology of the Xylariaceae broadly falls into two types depending on the spatial separation of the conidia. If several conidia are produced in rapid succession at the tip of

the conidiophore, the groups of scars will lead to the production of swellings on the conidiophore characteristic of the form genus Nodulisporium. This type of conidiophore is characteristic of most Hypoxylon species and also of Daldinia (Rogers, 1979a). Conidiophores which have spores that are blown out along their length at equal intervals of time, so that they are separated in space, are referred to as geniculate (Greenhalgh and Chesters, 1968). This conidiophore type is found in a small number of Hypoxylon species (those in section Papillata, subsection Primo-cinerea - see later) and species of Rosellinia. Within the family there are some species or genera that are exceptions, that is they do not produce conidiophores of either of these types. These include Poronia in which conidia are borne singly or in chains on filaments which themselves disarticulate at maturity (Greenhalgh and Chesters, 1968) and most species of Xylaria which produce sparingly branched persistent conidiophores in a tight palisade (Rogers, 1979a).

On such features as those described above many of the genera in the family were established in the eighteenth and nineteenth centuries. For example the genus Hypoxylon, one of the largest in the Xylariaceae, was established in 1791 by Bulliard (Miller, 1961). Since then the number of Hypoxylon species described in the literature has multiplied considerably. This is partly because undue emphasis on characters that vary in different environmental conditions has slowed the emergence of more appropriate criteria for classification. However, after considerable revision by several workers including Miller (1930, 1932, 1949, 1961), Martin (1967a,

1967b, 1968a, 1968b) and more recently Whalley and Greenhalgh (1973), Whalley (1976), Rogers (1979a) and Whalley and Edwards (1987), a coherent system has been developed which is illustrated by the schematic diagram in Figure 1.2 (adapted from Whalley and Edwards, 1987). This shows the lines of association between the closely related genera believed by Rogers (1979a) to be the central core of the family. Three genera, Anthostomella, Thamnomyces and Entonaema are not included, because although it is accepted that they belong to the group, their relationships with other xylariaceous genera are uncertain. Other authors including Cannon, Hawksworth and Sherwood-Pike (1985) and Eriksson and Hawksworth (1985) have listed the genera of the Xylariaceae and these systems depart in minor ways from that which is discussed below.

At the centre of the scheme in Figure 1.2 is the genus Hypoxylon which is itself comprehensively divided into four sections - Hypoxylon, Papillata, Annulata and Applanata by Miller (1961). Species in the section Hypoxylon have brightly-coloured stromata (usually a shade of red, purple or brown varying with environmental factors) which are leathery-woody in texture with umbilicate ostioles. The type of the section is Hypoxylon fragiforme; other members include Hypoxylon fuscum and the most cosmopolitan species Hypoxylon rubiginosum. By contrast species belonging to the section Papillata, in addition to having papillate ostioles, have stromata that are dark brown to black at maturity and have a carbonaceous texture. This section is further divided into subsections Papillata and Primo-cinerea on the basis that

immature stromata in the former contain red or yellow tints which may persist in maturity, while those of the latter are white to ash-grey. Hypoxylon multiforme is the type of subsection Papillata, while H. serpens is the type of subsection Primo-cinerea, which also includes Hypoxylon mammatum. Species of the section Annulata are found chiefly in the tropics and warm parts of the temperate zone. The most common species is Hypoxylon truncatum and this is the type of the section. Stromata that are flat to slightly convex, that do not vary much with the character of the substratum and do not separate into stromata with one or a few perithecia, characterize members of the section Applanata. Most of the species in this section have papillate ostioles and dark carbonaceous stroma, as illustrated by the type, Hypoxylon nummularium.

Miller's (1961) division of the genus Hypoxylon has been challenged by several workers including Whalley and Greenhalgh (1973) who placed greater emphasis on stromatal pigmentation as a taxonomic feature than ostiole type. Numerical analysis of a number of characters resulted in separate clusters of pigmented forms and non-pigmented forms (Whalley and Greenhalgh, 1973; Whalley, 1976). However, Rogers (1979a) still favours Miller's system as Greenhalgh and Whalley (1970) for example place H. nummularium in the same group as H. serpens and Hypoxylon confluens, but other observations indicate it is distantly related to these species, supporting Miller's wide separation of them (Rogers, 1979a). Nevertheless, the situation is not resolved, as Pouzar (1979) argues that H. nummularium does not belong to Hypoxylon, but should be placed in

Biscogniauxia, a genus he proposed to replace Nummularia, and to include species previously included in the latter by Miller (1961). Whether H. nummularium should be considered as a member of Hypoxylon or Nummularia (or a synonym of it) has been discussed by Jong and Benjamin (1971) but for convenience I shall continue to consider it an Hypoxylon species in Miller's (1961) section Applanata. I shall also refer to Nummularia rather than a synonym of it, because although it is strictly invalid due to the earlier homonyms of two angiosperm genera (Whalley and Edwards, 1985) most mycologists are familiar with this name.

The genus Daldinia is closely allied to Hypoxylon, being separable only on account of the concentric zonation of the ectostroma (Miller, 1932). Similarly, the genus Rosellinia, first described by De Notaris in 1884 (Martin, 1967b), shows great affinity with the subsection Primo-cinerea of Hypoxylon for a number of reasons. These include the possession of stromata that may be uni- or multiperitheciate (even within the same sample), similarity in stromatal structure, ascal plugs, spore shape and possession of the Geniculosporium anamorph (Rogers, 1979a).

In the same way the group of genera including Ustulina, its tropical equivalent Kretzschmaria, and Xylaria are more closely linked to the subsection Primo-cinerea than any other division of Hypoxylon. Closely allied to Xylaria is the genus Poronia which is delimited from it by its nail-shaped stromata and coprophilous habit (Dennis, 1978). Like Podosordaria and Hypocopra, Poronia

is adapted to coprophily by possession of ascospores with gelatinizing outer walls that help to adhere spores to herbage (Rogers, 1979a).

The genera Camillea and Nummularia are affiliated to the Applanata section of Hypoxylon and there is a perfect series forming a transition between the condition in the latter, where perithecia develop in the entostroma, just below the ectostroma, and that found in Nummularia where perithecia form at the base of the entostroma and so reach the surface by long ostioles (Miller, 1930, 1932).

Comparatively few connections between teleomorphs and anamorphs have been made in xylariaceous genera except in Hypoxylon (Rogers, 1979a). Martin (1967a, 1968a, 1968b) assigned the conidial states of Hypoxylon and other xylariaceous species to the hyphomycetous genera Sporothrix, Nodulisporium and Acrostaphylus. Jong and Rogers (1972) considered that Acrostaphylus was synonymous with Nodulisporium and that while the simple conidiophores of Xylaria and Rosellinia may be placed in the form-genus Sporothrix, the more highly branched conidiophores of Hypoxylon do not belong here. For the conidial state of H. serpens Greenhalgh and Chesters (1968) proposed the generic name Geniculosporium, and H. cohaerens could also be accommodated here. Other hyphomycetous genera in which the conidial states of xylariaceous fungi could be placed include Hadrotrichum, Periconiella, Virgariella and Rhinocladiella. Virgariella is allied to Nodulisporium and Geniculosporium and the

anamorphs of H. fuscum and H. cohaerens appear Virgariella-like (Greenhalgh and Chesters, 1968; Jong and Rogers, 1972). The simple conidiophore apparatus of Hypoxylon diathrauston approaches Rhinocladiella, a genus showing affinities to Geniculosporium, Nodulisporium and Sporothrix (Jong and Rogers, 1972). Few links between teleomorphs and anamorphs of other xylariaceous species have been made, presumably because they are rarely found together. However cultures of most species readily produce conidia (Rogers, 1979a) and even those that fail to sporulate may be induced to do so using wood pulp media (Whalley, 1979).

Certain characters of the Xylariaceae which used to be regarded as taxonomically important are now considered less useful, since they exhibit considerable variation in response to environmental factors such as climate, humidity, and host plants. Such characters include the size and form of stromata which, for example in Hypoxylon species are mainly dependent on the nature of the substratum, so that forms that are semi-globose when emerging from bark are thin and widely effused on hard decorticated wood (Miller, 1930). This is clearly illustrated by H. serpens, a species that varies in a number of features including the type of ascospore germ slit and ascus reaction with iodine, leading to the belief that H. serpens may be a complex of separate species (Rogers,1979a). Recently work by Pouzar (1985a, 1985b) and Petrini and Rogers (1986) has supported this belief and a group of closely related, but distinct species is referred to as the Hypoxylon serpens complex. With respect to stromatal characters, the most variable

species of Hypoxylon, H. rubiginosum is also the most common species of the genus in the world. Typically, the stroma is resupinate, but on Betula it develops through lenticels and may be mistaken for H. multiforme, whilst on small branches the small discrete hemispherical stromata may resemble H. fuscum (Miller, 1961).

Hypoxylon rubiginosum is not alone amongst the Xylariaceae in being cosmopolitan in its geographic distribution. Other species that are ubiquitous are H. serpens and U. deusta. However there are several species that are restricted to particular latitudes, for example H. mammatum and H. multiforme occur only in the Northern Temperate Zone (Miller, 1961), while genera such as Kretzschmaria, Phylacia, Camillea and Thamnomyces are strictly tropical (Martin, 1967a). A few species are confined to local geographic regions. These include Hypoxylon notatum in the southern United States of America (Miller, 1961), and Hypoxylon diathrauston which occurs on small branches of coniferous hosts at high elevations, usually above the snow level, in central Europe and whose ascospores will only germinate at near-freezing temperatures (Ouellette and Ward, 1970). Broadly speaking since the majority of xylariaceous species are wood-inhabitants, they tend to occur in the forested regions of the world, although Martin (1967a) reported Xylaria fioriana from the semi-arid scrubland of South Africa where rainfall is less than 45 cm y^{-1}.

(b) Ecological Characteristics

Fungi obtain organic nutrients from living or non-living substrata (resources) by one of three nutritional modes, biotrophy, necrotrophy and saprotrophy. In biotrophy readily assimilable soluble nutrients are absorbed directly from living cells, while in necrotrophy the living tissues are first killed by the fungus before it uses the constituent substrates, and in saprotrophy the fungus utilises non-living material killed by means other than the fungus itself. Regardless of the mode a fungus adopts, it may exhibit a range of behaviour from selectivity for a particular resource type/substratum, to occurrence in a wide range of substrata. Preference for a particular resource type, that is selectivity, may be in one of three overlapping forms, taxon selectivity, resource-unit restriction and habitat selectivity. Taxon selectivity is where living or dead material of particular groups of organisms, such as families or genera, are favoured. Resource-unit restriction is where a fungus is restricted within the boundary of the resource unit (Swift, 1976) for example fruit, petiole, twig or branch, whereas non-unit restriction denotes the situation where a mycelium grows freely between spatially separate resource units for example in litter. Habitat selectivity, which embraces both taxon selectivity and resource-unit restriction includes selectivity for particular habitat types, for example wood, dung or litter (Rayner, Watling and Frankland, 1985).

Most members of the Xylariaceae are probably obligate saprotrophs growing on wood, or less commonly on other lignin-

containing materials such as dung and litter, although some may be capable of necrotrophic parasitism on woody hosts. Species that select wood as a habitat may be divided into those associated with bark, those with decorticated and often well-decomposed wood and those that apparently show no preference. Hypoxylon fragiforme and H. fuscum are often associated with branches still attached to the parent tree and were considered by Chesters (1950) to be primary colonizers. Hypoxylon rubiginosum, H. serpens and Xylaria species are among the large number of species that can be considered as secondary colonists and they are often found on decorticated wood of fallen branches and stumps. In general, secondary colonists do not exhibit distinct host preferences (see below) and consequently attack all kinds of deciduous wood. Selectivity for dung is exhibited by the coprophilous genera Poronia, Podosordaria, Hypocopra and Wawelia (Whalley, 1985).

A few xylariaceous species occur in habitats that do not fall easily into the categories (wood, dung, litter) outlined above. These include Xylaria nigripes which grows on West African termite nests composed of masticated vegetable fragments (Dixon, 1965) and Hypoxylon hydnicolum in the United States which was reported by Miller (1961) as a mycoparasite of a species of Irpex - a wood decaying fungus which was itself established on Quercus.

As well as habitat selectivity, described above, most species also display resource-unit restriction, for example in wood-inhabiting species growth is restricted to within branches, logs,

stumps or occasionally whole trees. The initial colonization often occurs within living trees that have been predisposed by stress due to drought, fire, storm, pruning or other damage. In the late summer or autumn mature perithecia are found on dead wood or bark. These may be produced singly, or embedded in a stroma which may either disintegrate the following winter or persist as a sterile carbonaceous crust for several years. Some species continue to form fertile stromata for many years after the death of the infected part of the tree.

Certain species of the Xylariaceae are endophytes, that is they cause symptomless infections in healthy leaves and twigs of higher plants (Petrini and Carroll, 1981) and as such they are restricted to the blade and/or petiole of leaves. To date only a limited number of investigations of endophytic fungi have been made and the significance of fungal endophytes to their hosts is not clear. However, the indications are that xylariaceous endophytes are widespread and occur in a number of different plant taxa (Petrini and Petrini, 1985). They have been found in coniferous needles (Carroll and Carroll, 1978), in members of the Ericaceae (Petrini, 1984) and in lichens, bryophytes and pteridophytes. Some of the endophytic species occur throughout these groups, for example members of the H. serpens complex (Petrini and Petrini, 1985). Similarly, Nodulisporium species, including anamorphs of Daldinia species, and some Xylaria species have been isolated from a number of plants. These include ericaceous hosts (Petrini, 1984), evergreen shrubs in western Oregon (Petrini, Stone and Carroll,

1982) and Ulex europaeus and Ulex gallii - Nodulisporium species only (Fisher, Anson and Petrini, 1986). Other endophytic Xylariaceae, such as those in the genus Anthostomella and a few Hypoxylon and Rosellinia species, seem to be confined to members of a single plant family (Petrini and Petrini, 1985) and as such exhibit taxon selectivity.

Taxon selectivity is also a feature of the xylariaceous species that grow on wood. Dicotyledonous hardwood is especially favoured and many species exhibit a preference for particular host genera (Miller, 1961; Martin, 1967a). The significance of host selectivity is not certain and a species may be consistent in its preference throughout its distribution as in H. fragiforme which favours Fagus (Miller, 1961), or may alter hosts according to latitude. This seems to be the case in D. concentrica which occurs predominantly in England on Fraxinus while further north in Scotland it switches to Betula as its preferred host (Whalley and Watling, 1982). It has been suggested that this pattern corresponds with that observed in Europe (Whalley and Watling, 1982) where in France and Germany the fungus favours Fraxinus and Alnus (Winter, 1887; von Arx Müller, 1954) while in Norway Betula and Alnus are the usual host trees (Eckblad, 1969) and in Denmark Betula, Alnus and Populus are selected (Whalley and Knudsen, 1985, cited by Whalley, 1985).

Species that appear to be selective in their choice of hosts are not restricted to them, so that for example although D. concentrica favours Fraxinus as its host in England, it has been

found on at least sixteen different angiosperm species (Whalley and Watling, 1980, 1982). A few of the Xylariaceae occur on monocotyledons, for example, X. fioriana on Aloe ferox (Martin, 1967a) and H. rubiginosum on members of the Bambuseae (Miller, 1961). Even fewer species occur on coniferous wood such as H. diathrauston and Hypoxylon terricola which grows on coniferous needles and twigs in the leaf litter and was first found in Michigan, United States of America (Miller, 1961) and subsequently in the Atlantic Pyrenees under Cedrus atlantica (Candoussau, 1977). Although many Xylaria species are apparently not highly host selective (Rogers, 1983), some do demonstrate clear taxon selectivity as well as resource-unit restriction. For example Xylaria oxyacanthae which occurs on the rind of hawthorn (Crataegus) berries in Holland, Xylaria carpophila which is associated with beech (Fagus) cupules in litter (Whalley, 1985) and Xylaria magnoliae that inhabits the fruit of Magnolia species (Rogers, 1979b). The wide variety of host preference and geographic distribution in the Xylariaceae are well represented merely by the genus Hypoxylon, examples of which are given in Table 1.1.

The wide geographic distribution of the Xylariaceae and their predominant association with dicotyledonous angiosperms have led to speculation as to their origins. The fossil record is poor; the first definite appearance of a xylariaceous fungus is in the early Cenozoic long after angiosperm evolution and diversification (Tiffney and Barghoorn, 1974). However it seems probable that the ancestors of the present day Xylariaceae inhabited the pre-

angiospermous flora - gymnosperms and/or ferns, and later the early angiosperms, which are believed to have originated and evolved in xeric or seasonally dry areas. These conditions could have exerted selective pressures favouring the development of fungi that could survive periods of drought, and eventually the xylariaceous ancestors may have become extinct in competition with their better adapted descendants. Such adaptations may include features that are apparent in contemporary Xylariaceae, such as perithecial stromata that can tolerate severe desiccation without impairing ascospore development and dispersal, once moist conditions return (see below). Following their evolution on angiosperms in dry areas, the Xylariaceae probably continued to evolve on dry and other marginal sites. Subsequently the opportunity arose to inhabit the species-rich sub-tropical and tropical forests, where the greatest numbers of genera and species exist today. Coprophilous genera probably evolved from the lignocellulolytic associates of woody hosts, with the evolution of herbivorous mammals whose dung provided an ideal substratum for colonization by plant-inhabiting fungi (Rogers, 1979a).

The Xylariaceae are typical of many Pyrenomycetes in their ability to tolerate long periods of drought and to this end they are variously adapted (Rogers, 1979a). In some species, for example Hypoxylon udum and Anthostomella, perithecia are reduced and immersed in the substratum to protect against desiccation, while in others, such as many Hypoxylon species and Daldinia, the large sessile stromata may minimize water loss. The dung itself is

thought to provide the stromata of coprophilous Hypocopra with the advantages of a massive stroma (Rogers, 1979a) and Wawelia octospora seems to favour conditions of low relative humidity (Minter and Webster, 1983). Nummularia and applanate species of Hypoxylon appear to be specially adapted to tolerating drought, as they have a bipartite stroma which develops within the bark of host trees. The inner and outer regions of the stroma are separated by a gelatinizing layer that swells, forcing the two regions apart and rupturing the bark which is shed, together with the outer regions, as the stroma no longer requires protection from excessive water loss (Rogers, 1979a).

Other adaptations to cope with drought include rapid germination of ascospores in water and spore discharge over long periods, usually at night in wet conditions (Rogers, 1979a). This was found for H. rubiginosum and H. fragiforme, in which the maximum discharge period coincides with that of highest rainfall and relative humidity (October) (Hodgkiss and Harvey, 1969). In dry periods there is no discharge in H. rubiginosum, but rain only lasting one hour induces an immediate spore discharge response (Hodgkiss and Harvey, 1970). Similarly Pady, Kramer and Clary (1967) found Hypoxylon species discharge their spores at night in a typical diurnal pattern, although spores can be released into the air in daytime following a period of rainfall. Contrasting with this is spore release in D. concentrica, one of the few ascomycetes in which discharge can be initiated and sustained without direct wetting. The water required is obtained from the reserve in stromal

tissue which decreases in density as the discharge period progresses. It is possible that it is a result of its unusual water relations that D. concentrica has a summer discharge period, unlike other species which tend to have a peak discharge in the more humid conditions of autumn (Ingold, 1971).

Although most members of the present-day Xylariaceae are saprotrophs, some are necrotrophic parasites and are considered to be pathogenic. Probably the most familiar of these is H. mammatum which causes a canker disease of Populus species in the Lake States of America. Here aspen (Populus tremuloides) is grown commercially on a large scale for pulpwood and Hypoxylon canker is the most serious fatal disease of the tree, resulting in losses, estimated in 1971, as being in excess of four million dollars per year at harvest (Marty, 1972). Another species of economic importance is Ustulina deusta, sometimes referred to as Ustulina zonata in the tropics. This was first recorded as a parasite resulting in commercial losses by Petch in 1907, as it was found to be the cause of "low-country root disease" of tea. Later, in 1914, Brooks found that it was the causal agent of dry collar rot of rubber (Hevea brasiliensis), which was of economic importance particularly in Malaya (Wilkins, 1934). In temperate areas of the world U. deusta causes butt rot in lime (Tilia vulgaris), elm (Ulmus species) and beech (Fagus sylvatica) (Wilkins, 1936, 1939, 1943) and in the United States of America has also been recorded as common on certain Acer species, including red maple and sugar maple (Campbell and Davidson, 1940). Recently Prljincevic (1982) has drawn

attention to the significance to the forest economy of U. deusta (his Hypoxylon deustum) in the Balkans, where it has caused serious damage to mature beech trees.

Closely related to Ustulina and possibly a variant of it, is the genus Kretzschmaria (Rogers, 1979a) and Kretzschmaria clavus has been shown to be the cause of root rot of macadamia (Macadamia integrifolia) in Hawaii (Ko, Kunimoto and Maedo, 1977; Ko, Ho and Kunimoto, 1982). Root rots are also caused by species of Rosellinia, for example Rosellinia necatrix which causes a white root rot of fruit trees and Narcissus species (Mantell and Wheeler, 1973), and Rosellinia desmazieresii which has recently been found to attack the roots and cause ring-dying of creeping willow (Salix repens) at Ainsdale Sand Dunes Nature Reserve, southwest Lancashire (Barrett and Payne, 1982). Similarly Xylaria species cause root rots of angiosperms, in particular Xylaria mali which causes black root rot of apples (Malus). Hepting (1971, cited by Rogers, 1979a) noted that apples and allied rosaceous genera are also prone to canker disease caused by Nummularia discreta and that cankers on sycamore (Platanus) species may result from infection by H. tinctor. In Portugal the cork oak (Quercus suber) is susceptible to trunk disease with which Hypoxylon mediterraneum is associated (Rogers, 1979a). Xylariaceous species associated with leaf diseases are less common than those associated with root rots and stem cankers, although Rosellinia herpotrichioides causes needle blight on coniferous trees such as hemlock (Tsuga species), Douglas fir

(Pseudotsuga menziesii) and Sitka spruce (Picea sitchensis) (Whalley, 1985).

The xylariaceous pathogen H. mammatum, has been extensively studied, probably because of the severe losses it causes. The disease was first reported in 1921 and the pathogen was found to be native to North America, although recently it has been recognized in Europe, and there is evidence to suggest that it may have been present in the Alps for at least 25 years (Pinon, 1979). Although the host range of the fungus includes woody plants in the genera Acer, Alnus, Betula, Fagus and Ulmus among others, it is generally considered to be a disease-inducing pathogen of Populus alba, Populus tremula, Populus tremuloides and Populus grandidentata (Manion and Griffin, 1986).

Bark infected with H. mammatum is characteristically mottled black and white, and in time becomes totally blackened and cracked in a chequer-board fashion, giving rise to a diffuse canker, which may coalesce to girdle and ultimately kill the tree. One year after initial infection, the asexual stage of the fungus is apparent causing the outer bark layer to blister and peel back, and two to three years after infection, the perithecial stromata appear (Manion, 1981). The exact site and conditions under which infection takes place are still not certain, although several studies suggest the involvement of insect wounds, such as galls and galleries of Saperda species, including Saperda concolor (Manion, 1975) and Saperda inornata (Anderson, Ostry and Anderson, 1976; Anderson and

Ostry, 1983) and oviposition wounds of cicada species (Anderson, Ostry and Anderson, 1979). Branch stubs and axils have also been implicated (Manion, 1975; Ostry and Anderson, 1979) as well as low bark moisture levels (Bier, 1961) and physical and chemical properties of the soil relating to moisture (Bruck and Manion, 1980). The role of environmental conditions in disease development is implied by the discovery that Hypoxylon infected aspens die more rapidly under shaded conditions (Anderson, 1972).

Hypoxylon mammatum is probably the first xylariaceous species that has been investigated with respect to its variability and apparent host preference (Rogers, 1979a). Single ascospore isolates of the fungus are extremely variable in their cultural morphology, growth rates, conidial production and virulence to aspen (Bagga and Smalley, 1973; French and Manion, 1975; Griffin, et al., 1984). Only 5 - 6% of isolates produce conidia in culture (Bagga and Smalley, 1973) and these produce fewer cankers in clones of P. tremuloides than "mycelial type" isolates (French and Manion, 1975). Clones of P. tremuloides themselves can vary in susceptibility to canker development (French and Manion, 1975; French and Hart, 1978; Griffin et al., 1984), although other plant species do not appear to be so susceptible. This was shown by Schipper (1978) when he tested 27 plant species for sensitivity to a phytotoxin produced in culture by H. mammatum and found that only Populus species were highly sensitive. Other workers, including Pinon (1984) and Griffin and Manion (1985), have found that

different poplar clones themselves vary in their reaction to this toxic substance.

The phytotoxin had previously been found to be heat resistant, water-soluble and to elicit extensive bark necrosis when applied to unwounded aspen (Bagga and Smalley, 1973). Schipper (1975, 1978) suggested that the toxin appeared to interfere with wound-healing by inhibiting callus formation during the infection stage of parasitism. Attempts to isolate and purify the toxic substance, referred to as mammatoxin, have shown that several compounds, separable by chromatography and partitioning in organic solvents, are produced that cause responses to bioassays (Schipper, 1978; Griffin, Ehrenshaft and Manion, 1980, cited by Manion and Griffin, 1986; Stermer, Scheffer and Hart, 1984). The kinds of compounds produced differ between fungal isolates (Griffin, Ehrenshaft and Manion, 1980) and the quantitites according to culture age (Stermer, Scheffer and Hart, 1984). To date there has been no success in purifying and identifying any toxic metabolite (Manion and Griffin, 1986).

Other members of the Xylariaceae have also been found to produce toxins and other metabolites and although only a limited number of studies have been made on this aspect of their biology, they are sufficient to indicate the variety of chemicals produced. With regard to toxins, several species of Hypoxylon and Xylaria are known to produce cytochalasin E, a toxic material which may provide

the fungus with a competitive advantage in soil and litter (A.J.S. Whalley, pers. comm.). Rosellinia necatrix also produces this compound, along with rosellinic acid and diketopiperazines (Anderson, Edwards and Whalley, 1983).

In 1979 Edwards and Whalley carried out a chemotaxonomic survey of metabolites produced in culture by various members of the genus Hypoxylon and this resulted in the isolation of two new isomeric γ-butyrolactones produced by H. serpens. Later Anderson, Edwards and Whalley (1982) investigated "Barron's strain" of H. serpens and found that it yielded another new butyrolactone, an odoriferous oil referred to as "serpenone". By contrast it was found that Hypoxylon chestersii, which is closely related to H. serpens, did not produce lactones, but instead yielded a new allenic ether - chestersiene - the first aromatic ether recorded in nature (Edwards, Anderson and Whalley, 1982). Similarly a compound referred to as punctatin A, a sesquiterpene, produced by the only Poronia species recorded in Britain - Poronia punctata - had not previously been found in nature (Anderson et al., 1984a). This fungus produces in culture a family of sesquiterpene alcohols, including punctatin A, which is the major component, and several isomers of it, one of which has been characterized as punctatin B. A third compound, punctatin C, which has a novel tetracylic skeleton, but is closely related biosynthetically to the other two, occurs in small amounts (Anderson et al., 1984a, 1984b).

In addition to the sesquiterpenes, P. punctata is also believed to produce antibiotics, as it is known to be antagonistic towards other fungi (A.J.S. Whalley, pers. comm.). Other xylariaceous fungi also produce antibiotics, for example Nummularia broomeiana produces pyrenophorin as well as dihydroisocoumarins (Anderson, Edwards and Whalley, 1983). Dihydroisocoumarins, with 5-methyl mellein as a common constituent, are in general associated with Hypoxylon and Nummularia species (Whalley and Edwards, 1987) while succinic acid derivatives have been found to be produced by members of Xylaria and its close relatives such as U. deusta (Anderson, Edwards and Whalley, 1985).

Some work has been done regarding the pigments of the xylariaceous stroma. For example Greenhalgh and Whalley (1970) extracted stromatal pigments of some British species of Hypoxylon and examined the pigment patterns produced by thin-layer chromatography. They found that species that belonged to the same section of the genus, produced similar chromatograms and that most species showed constant and characteristic band patterns, although some, such as H. rubiginosum, produced varied ones. As the latter species grows on a variety of host substrata, it was suggested that either different band patterns correlated with different host substrata, or that the species was divided into different chemical races. Further work by Whalley and Greenhalgh (1971) indicated that the latter is the case and they proposed that in H. rubiginosum races may exist which differ not only in their pigment characters, but perhaps also in physiological and biochemical ones which are

adapted to different substrata. The chemical nature of the pigments extracted during the survey was not elucidated, but they were not of a quinone nature and none of them were strongly phenolic. By contrast stromata of D. concentrica contain dihydroxyperylene quinone (Rogers, 1979a) which is derived from chromagen (Miller, 1961) and the brown-red pigment layer covering H. fragiforme stromata contains a mixture of four (+)-mitorubrin derivatives (Steglich, Klaar and Furtner, 1974). Some xylariaceous species produce pigments in culture, although their chemical nature has rarely been investigated. Daldinia concentrica is an exception - it has been found to produce naphthalene derivatives, dihydroxyketones and the chromanene and chromone in culture (Anderson, Edwards and Whalley, 1983) while staining the medium "dark-fawn" (Cartwright and Findlay, 1942). Other species that have been examined in this respect include H. fragiforme, Hypoxylon haematostroma and Hypoxylon venustuissimum which produce ramulosin, iso-ochracein and mellein (Anderson, Edwards and Whalley, 1983).

All members of the Xylariaceae have the capacity to degrade lignin and cellulose, that is to cause a white rot (Rogers, 1979a) although their ability to do so may differ between species. Daldinia concentrica for example does not actively decompose lignified material (Cartwright and Findlay, 1942) and H. rubiginosum is known to produce greater quantities of cellulase than any other Hypoxylon species (A.J.S. Whalley, pers. comm.). Sutherland and Crawford (1981) examined degradation of the lignin and glucan components of lignocellulose by Hypoxylon cohaerens var.

microsporum, H. serpens, P. punctata, Rosellinia limoniispora and Xylaria hypoxylon. All the species converted a significant amount of the lignin component to carbon dioxide, although a higher percentage of the glucan component was degraded to carbon dioxide and water-soluble products. Rosellinia limoniispora degraded the highest percentage of each. Wicklow, Detroy and Adams (1980) studied the modification of lignin and cellulose components in wheat straw by fungal colonists of ruminant dung. They found that although P. punctata had one of the slowest growth rates of the species examined, it was the most effective at degrading cellulose.

In wood the Xylariaceae cause a decay that is similar to that produced by Basidiomycotina, although the rate of decay is often slower. Merrill, French and Wood (1964) compared the relative ability of several xylariaceous species to decay oak (Quercus rubra) and aspen (Populus tremuloides) wood with that of Lenzites trabea and Coriolus versicolor and showed that in general the former required 32 weeks to cause as much decay as the Basidiomycotina had caused in 3 to 4 weeks. However, the xylariaceous fungi were still causing increasing weight losses at 32 weeks and the maximum amount of decay caused was not determined.

Daldinia concentrica is considered to be a primary colonizer establishing in living or recently dead tissue by latent invasion (Boddy, Gibbon and Grundy, 1985) a process that is also believed to explain the dramatic increase in Hypoxylon atropunctatum canker on oaks (Quercus spp.) in the southern United States of America,

following the drought of 1980 (Bassett and Fenn, 1983, 1984). The process of latency will be discussed further below. As mentioned earlier, other primary colonizers, preceding basidiomycete decayers, are H. fragiforme and H. fuscum while H. serpens and Xylaria species are considered to be secondary colonizers (Whalley, 1985). Some species such as H. multiforme are successful as both primary and secondary colonizers (Taligoola and Whalley, 1976).

As both primary and secondary colonizers, these fungi will probably at some time during their existence in the wood, come into direct contact with other fungal species. Until recently there have been few studies regarding interspecific interactions involving members of the Xylariaceae, but Boddy, Gibbon and Grundy (1985) reported that D. concentrica was combative in culture, replacing most of the wood-decaying species it was paired against, although under different gaseous conditions the outcome was modified. Another aspect of the biology of the Xylariaceae that has been neglected, is their intraspecific mycelial interactions and their population structure, that is the distribution of genotypes within and between hosts. Since population structure directly reflects patterns of spread and establishment, a knowledge of it may provide insights into ecological strategies, possible life histories and perhaps reasons for host selectivity (Rayner and Boddy, 1986).

The first work of this nature on the family was reported by Dowson (1982) and provided the basis for the present study. It will be discussed below after a brief outline of relevant background

information regarding intraspecific interactions and fungal population biology.

ii. Population Biology

(a) Mating and somatic incompatibility

Work in the 1970s on a variety of white-rotting basidiomycetes in hardwood stumps and logs revealed that a system existed by which genetically distinct mycelia (individuals) were delimited within natural populations (Rayner and Todd, 1977, 1979). This agreed with similar results from other fungi (such as the work of Barrett and Uscuplic (1971) on Phaeolus schweinitzii) suggesting a departure from the previously accepted unit mycelium concept arising from the work of Buller (1931) and supported by Burnett and Partington (1957). This concept assumed that genetically distinct mycelia of the same fungus species, meet in a substratum and become physiologically and ecologically unified into a mosaic, within which individual genotypes cannot be recognized as separate entities. Rayner and Todd (1977, 1979) however, observed that wood decayed by a variety of Basidiomycotina contained decay columns separated by narrow, relatively undecayed regions and that isolates derived from either side of such zones, even if they were the same species, were frequently antagonistic. They concluded that genetically distinct mycelia were clearly delimited and functioned independently as individuals within natural populations and that Buller's observations were as a result of the fungus he worked with, Coprinus sterquilinus, being homomictic, so that spores were likely to be genetically similar or identical.

Since the 1970s there have been a number of fungal population studies, including some on Ascomycotina which have largely followed the same principles applied to Basidiomycotina, although the genetic mechanisms which generate and maintain variation in heterothallic species of the two groups - the mating systems of sexual outcrossing - are fundamentally different (Rayner and Boddy, 1986). A discussion of these mating systems and the basis of recognition of individual genotypes in the population as explained by Rayner *et al*. (1984) and Rayner and Boddy (1986) will follow, after which there will be a brief outline of relevant work on the Ascomycotina to date.

The mating system that operates in the Ascomycotina may be described as dimictic, that is it is as though mating is controlled by a single, biallelic locus (Burnett, 1975, 1976). In the population there are two "mating types" which are compatible with one another and will successfully produce viable ascospores, however each type is self-sterile. This type of mating system enforces *outcrossing*, that is fertilization between genetically different individuals, but does not intrinsically promote *out-breeding* (fertilization between non-sib related lines only) because only two types of mating-compatible individual exist in the population as a whole.

The mating system in the Basidiomycotina (except Hemibasidio-mycetes) is quite different and is referred to as diaphoromictic. Here mating types are determined by one, two or exceptionally three un-linked *multiallelic* mating type factors, so that sib-related

progeny from a single fruit body have two, four or eight mating specificities respectively (the mating systems are thus referred to as bipolar, tetrapolar or octopolar). In the breeding population as a whole, outbreeding is promoted (Burnett, 1975, 1976) because numerous mating-compatible types occur as a result of the multi-allelic nature of the mating factors.

Outcrossing Ascomycotina and Basidiomycotina also differ in that in the former plasmogamy often occurs between specialized sexual organs and the asogenous hyphae are the only part of the mycelium that becomes heterokaryotic for mating type (that is contains the two types of nuclei of different mating type). By contrast in the Basidiomycotina protoplasmic fusion occurs between vegetative homokaryotic mycelia which develop from germinating basidiospores and this results in a stable, independent, heterokaryotic mycelium.

Other ways by which ascospores and basidiospores are produced result in clonal progeny sets. This is because there is no opportunity for recombination as conjugation between complementary mating types does not occur. Such processes include homomixis (primary homothallism - i.e. self fertility of the haploid homokaryon), homoheteromixis (secondary homothallism, i.e. production of binucleate sexual spores that are heterokaryotic for mating type) and amixis or apomixis (homokaryotic fruiting and mitotic production of basidiospores). Homomixis does not prevent outcrossing in the Ascomycotina because they have sex organs, but

it does appear to do so in Basidiomycotina, for example in Stereum sanguinolentum (Rayner and Turton, 1982), as a result of somatic incompatibility (see below) between separate homokaryotic lines. Mycelial fragmentation and conidiogenesis, both asexual processes, also produce clonal offspring.

Normal outcrossing is not the only process resulting in variation. Alternatives are somatic mutations, the possibility of parasexuality (although this is limited as somatic incompatibility restricts vegetative hyphal fusions to near isogenic lines) and developmental variation. With regard to the latter, switches between different morphological modes may be a result of differential gene expression.

As mentioned earlier it is now accepted that genetically different individuals in ascomycete and basidiomycete populations can often be delimited by a self-non-self recognition mechanism referred to as somatic incompatibility. This mechanism is distinct from, but complementary to, mating compatibility discussed above and in nature the two systems operate in a delicate balance against one another. The basis of somatic incompatibility is that adjacent mycelia will reject one another if they differ genetically at their polygenic or multiallelic somatic incompatibility loci. The rejection is expressed as a reaction zone between the mycelia which can vary considerably, but typically contains a sparse mycelium of disrupted hyphae and may be accompanied by accumulation of pigment in the medium or in the hyphae themselves.

Ascomycotina and Basidiomycotina differ in that in the former somatic incompatibility is typically expressed between primary homokaryotic mycelia which comprise the predominant vegetative phase, whereas it is expressed between secondary heterokaryotic mycelia in outcrossing Basidiomycotina and between different homokaryotic lines forming clonal populations in non-outcrossing Basidiomycotina. Homokaryons of outcrossing Basidiomycotina sometimes express somatic incompatibility if they are mating type incompatible, but if they are mating type compatible, it is not manifested at all, or is only revealed temporarily before it is overridden to allow a stable heterokaryon (secondary mycelium) to form.

The process of override probably involves several mechanisms concerning the migration and stable association of complementary nuclei. A working model proposed by Rayner et al. (1984) involves access migration, acceptor migration and stabilization. Access migration allows donor nuclei to pass into an acceptor mycelium at a rate determined by the genetic relationship between donor and acceptor and may result in migration patterns that are asymmetric or unilateral. It is responsible for inhibition of colony extension, abnormal branching patterns and proliferation of hyphal fusions via which donor nuclei may migrate laterally through a mycelium. Nuclei may also migrate via pre-existing hyphae, which is referred to as acceptor migration and can only occur following access. The migration rate here is determined solely by the acceptor. The final step is stabilization in which the two

complementary nuclei become stably associated in a single heterokaryotic mycelium.

In wood, as discussed above, narrow relatively undecayed interaction zone lines, as seen in cross-section, usually mark the boundaries of decay columns occupied by individual genotypes of the same species that are somatically incompatible (Rayner and Todd, 1977, 1979). It is important to distinguish these zones resulting from intraspecific antagonism from zone lines resulting from other causes, such as the interaction between two different species (interspecific antagonism), a host reaction to fungal invasion of living tissues and the laying down of pseudosclerotial plates (Rayner and Todd, 1979, 1982). Pseudosclerotial plates (PSPs) are sheets of closely interwoven hyphae which are typically pigmented and pseudoparenchymatous (Campbell, 1934; Lopez-Real, 1975). They may be laid down by a single colony either in the course of its normal growth in wood, or in response to environmental stimuli such as high temperatures, fluctuating moisture levels or desiccation. Zone lines due to PSPs are the most likely to be confused with those caused by intraspecific antagonism. However, the latter are usually paler (commonly pale brown), broader and more diffuse than PSPs and consist of undecayed wood rather than sheets of mycelium. Further, they generally delimit longitudinally extensive columns of decay, whereas PSPs produced by a single colony may be irregular in distribution and often either surround numerous small adjacent pockets of decay, or are produced adjacent to exposed wood surfaces. Sometimes PSPs can be produced between interacting

colonies of decay fungi and this may result in two parallel PSPs occurring on either side of the interaction zone (Rayner and Todd, 1982).

In the 1930s observations of zone lines were recorded in timber decayed by both Basidiomycotina and Ascomycotina, including several xylariaceous species such as Ustulina vulgaris, H. fragiforme, D. concentrica and Xylaria polymorpha. These black lines associated with xylariaceous species were invariably considered to be marginal zones of the entostroma in the substratum (Campbell, 1932), that is PSPs.

(b) Relation of the genetic structure of populations to colonization processes.

Analysis of genotype distribution within natural substrata may indicate the manner in which a fungus arrives at or on a resource (that is whether it arrives as genetically identical, or genetically different propagules, or by mycelium) and the invasion and colonization strategies which it adopts (Rayner and Boddy, 1986).

A characteristic feature of arrival via propagules is that colonization is effected from localized foci where germination has occurred. If the propagules are genetically identical, for example asexual spores produced by a single mycelium, or ascospores/basidiospores in non-outcrossing species, there is the possibility that mycelia growing from separate colonization foci will coalesce into a single unit. However, if the propagules are genetically

different, for example ascospores/basidiospores in outcrossing species, the resulting mycelial individuals may be expected to occupy discrete areas bounded by somatic incompatibility reactions (Rayner et al., 1984). By contrast, colonization following arrival by mycelium is not necessarily localized into separate foci, and water and nutrients can be imported, instead of only having the use of what is available in the immediate vicinity. As a result, species adopting this strategy have a greater energy of invasion, or inoculum potential, and so single genotypes may come to occupy considerable volumes of individual resource units, as well as large areas of ground. Arrival by mycelium may be effected either by diffuse mycelium, as probably occurs in litter fungi that are non-unit restricted, or via specialized migratory organs such as cords and rhizomorphs. These organs are characteristic of many litter inhabiting Basidiomycotina that exploit discontinuous resources such as roots, fruits or wood pieces (Rayner, Watling and Frankland, 1985).

With respect to wood, fungi may establish in the standing tree or in fallen timber (Rayner, Boddy and Dowson, 1987). Colonization is often initiated in the former despite the conditions imposed in functionally intact sapwood being considered unfavourable for mycelial growth (Rayner, 1986; Rayner and Boddy, 1986). These conditions include high water content, corresponding restricted aeration and a gaseous phase which is normally high in carbon dioxide and low in oxygen (Boddy and Rayner, 1983). Further, access to the standing tree is impeded by a range of physical and chemical

barriers that ensure proper functioning of the tree, and for colonization to occur these must be overcome, circumvented or tolerated. To this end five colonization strategies have been postulated. These are unspecialized opportunism, active pathogenesis, specialized opportunism, heartrot and desiccation tolerance. It is important to note that these strategies are part of a spectrum of behaviour and organisms may exhibit combinations of the different strategies, either at one and the same time, or at different times during their life cycle (Rayner, Boddy and Dowson, 1987).

Unspecialized opportunism is a strategy exhibited by a fungus that gains access to sapwood which is made suddenly available for colonization by injury or rapid death of the bark. Such a fungus is taking advantage of the alleviation of the unfavourable conditions in functional sapwood. By contrast, a fungus that itself alleviates these hostile conditions by killing living tissues and destroying pit membranes, is colonizing via active pathogenesis. Specialized opportunism may be displayed by fungi that are specialized in their ability to tolerate and initially establish themselves in the stressful conditions imposed in functional sapwood, but they are opportunists, as they then capitalize on subsequent alleviation of the stressful microenvironmental conditions arising through factors other than those related to their own activities (Rayner, Boddy and Dowson, 1987). This possible natural colonization process is referred to as latent invasion (Rayner, Watling and Frankland, 1985). This is suspected when an extensive mycelium occurs in a

resource and appears to have developed more rapidly than would be expected by normal patterns of mycelial spread from a colonization court. It is believed that a propagule that gains entry to a living tree or branch, via a minor discontinuity such as a leaf, twig scar, or lenticel, produces several separate mycelial units, such as buds, cells, mycelial fragments, or oidia (Cooke and Rayner, 1984) in response to the microenvironmental conditions present that are unfavourable to filamentous growth. The mycelial units could then be disseminated in the sap stream so that the fungus may be extensively present in a tree or branch, although not overtly so, under conditions imposed by functional sapwood. Later, perhaps following drought or mechanical injury which alleviates the stressful microenvironmental conditions in the tree, the mycelial units could revert to filamentous mycelial development and cause typical decay associated with loss of sapwood function (Boddy and Rayner, 1983). There is strong circumstantial evidence for this hypothesis of latent invasion, although it still requires substantiation (Boddy, Gibbon and Grundy, 1985; Rayner, Boddy and Dowson, 1987).

Heartrot fungi circumvent the problems of colonizing sapwood by growing in the heartwood where living cells are absent or rare, whilst fungi that exhibit a tolerance to desiccation are believed to be able to colonize standing trunks or branches which have lost their bark or sapwood, which results in fluctuations in the moisture content of the underlying wood. The latter may completely dry out and fungi tending to adopt this colonization strategy

presumably survive these conditions in a dormant state, yet possess the ability to resume functioning rapidly when wetted.

Once the standing tree has been colonized by fungi, the tree begins to decline. Unspecialized opportunists, initially confined to the damaged area, may extend into uncolonized wood. Opportunistic fungi may also colonize as the conditions that are unfavourable to their growth are alleviated by pathogens. The extent of colonization and tissue death expands. Heartrot fungi encroach outwards and come into contact with sapwood inhabitants and the number of interfungal interactions increase. Later, truly combative fungi (i.e. fungi able to defend domain they already possess and to acquire domain from previous residents - see below) that may arrive by airborne propagules, establish themselves and replace the primary colonizers. Ultimately the intense decay caused by the fungal communities may hasten the fall of branches and windthrow of trunks.

Fallen timber rarely arrives on the woodland floor in an uncolonized state and here fungi that actively decompose wood and litter, primarily Basidiomycotina, either exhibit the capacity to defend domain which they have captured before, or soon after fall (acquired by "primary resource capture"), or the ability to take possession of domain from previous residents (a process referred to as "secondary resource capture") (Cooke and Rayner, 1984; Rayner and Webber, 1984; Rayner, Boddy and Dowson, 1987). The difference between the defensive and attacking strategies is often reflected in a further distinction between two types of behaviour - that of

resource-unit restriction and non-restriction (Cooke and Rayner, 1984; Rayner, Watling and Frankland, 1985). As mentioned above, mycelia of fungi that exhibit the former behaviour are confined to individual resource units such as twigs, petioles, fruits and branches, and often establish themselves prior to fall perhaps via latent invasion, or they arrive after fall by airborne propagules. By contrast, mycelia of non-unit restricted fungi have the ability to colonize discontinuous resource units on the woodland floor and arrive and migrate between those units as mycelium (e.g. mycelial cords and rhizomorphs). These fungi are highly combative and ultimately, they frequently replace unit-restricted fungi.

In managed woodland or forest, timber that has been cut or felled presents further opportunities for fungal establishment. Usually this is a previously uncolonized resource. Fungi that initially establish themselves here often possess a ruderal ecological strategy, that is they rapidly arrive (often via propagules), capture the resource and become committed to reproduction before they are replaced by more combative competitors. This is in contrast to the fungi which colonize standing or fallen timber (mentioned above) that possess stress-tolerant or combative ecological strategies and which generally have a long individual life span and slow or intermittent commitment to reproduction. Examples of fungi exhibiting stress-tolerant characteristics are those which colonize by latent invasion or desiccation tolerance, whilst Basidiomycotina on the woodland floor may display combative features. As for the five colonization strategies described for fungal establishment in the

standing tree, these ecological strategies, ruderal, stress-tolerant and combative are part of a spectrum. Individual organisms may exhibit combinations of the different strategies at a single moment in time, or at different times during their life cycle (Rayner, Boddy and Dowson, 1987).

(c) Previous studies of genetic structure of ascomycete populations

There have been some studies of Ascomycotina involving analysis of genotype distribution in field populations and somatic incompatibility. A major study was that of Aspergillus nidulans reviewed by Croft and Jinks (1977). Field isolates from a range of localities were found to fall into 19 heterokaryon compatibility (h-c) groups where members of any one group formed heterokaryons with each other, but not with any member of any other group. Between groups there was wide variation in characters such as growth rate and penicillin titre, but there was little variation within each group, leading to the suggestion that each group was of clonal origin even though sometimes a group's members were geographically widely scattered. The latter situation is probably a result of long range dispersal of asexual spores. Heterokaryon formation was found to be under nuclear control involving a polygenic heterogenic system so that heterokaryon incompatibility arises between isolates if they have different alleles at one or more of a large number of het loci. The role of this system in maintaining the h-c groups is probably to delimit individual genotypes (Todd and Rayner, 1980).

Other studies of somatic incompatibility in Ascomycotina have included detailed work (discussed below) on the pathogens

Cryphonectria parasitica (formerly Endothia parasitica) the cause of chestnut blight (Anagnostakis, 1984b) and Ophiostoma ulmi (formerly Ceratocystis ulmi) the causal agent of Dutch elm disease (Brasier, 1984). Heterokaryon incompatibility has been demonstrated in Neurospora crassa (Garnjobst and Wilson, 1956; Mylyk, 1975, 1976; Perkins, 1979) and a non-allelic vegetative incompatibility system has been described for Podospora anserina (Labarerere, Begueret and Bernet, 1974). It appears that Monilinia fructicola may also have a vegetative incompatibility system as indicated by mycelial interaction zones between paired ascospore isolates (Sonoda et al., 1982). A similar phenomenon was reported between certain field isolates of the coprophilous ascomycete Ascobolus immersus and here vegetative incompatibility was believed to act as a barrier preventing genetic exchange between races of the fungus (Meinhardt, Koch and Esser, 1984).

The system of somatic incompatibility in C. parasitica and O. ulmi is similar to that described in A. nidulans. Field isolates of these fungi fall into different vegetative (somatic) compatibility (v-c) groups, within which isolates are compatible and between which they are incompatible.

Seventy three v-c groups of C. parasitica have been identified from 258 North American field isolates (Anagnostakis, 1984b), while most of 141 European strains that were tested fell into 22 v-c groups (Grente, 1981, cited by Anagnostakis, 1984b). A comparison of the diversity of v-c groups in Connecticut and Europe revealed that v-c groups were more diverse in the former so it was predicted

that one in every three isolates obtained would belong to a new v-c group, while in Europe a different type would only appear once in every six isolates. The 165 C. parasitica isolates from Connecticut fell into 67 v-c types, 38 of which were found only once and only three were found more than ten times (Anagnostakis, Hau and Kranz, 1986).

"Super v-c groups" occur in the aggressive strain of O. ulmi to the extent that 40% and 60% of isolates from a worldwide sample of the North American race (NAN) and Eurasian race (EAN) respectively, belonged to the same v-c group; the remaining isolates each belonged to a different v-c group. Isolates from a worldwide sample of the other reproductively isolated sub-population of the fungus - the non-aggressive strain - almost all belonged to a different v-c group except in North America where a super group may also exist (C.M. Brasier, pers. comm.). The super groups of the NAN and EAN are believed to arise as a result of the chance dispersal and spread of a particular group during the current epidemic (Brasier, 1984).

The larger number of v-c groups in both C. parasitica and O. ulmi suggest that vegetative incompatibility is under multiallelic and/or polygenic control. In O. ulmi polygenic control was implicated by a backcross experiment involving the elimination of vegetative incompatibility factors. In culture paired O. ulmi isolates produced five types of vegetative interactions - wide (w), narrow (n), line (l), line-gap (lg) and compatible (c) and these

were considered to correspond with strong vegetative incompatibility through weak incompatibility to compatibility. For the experiment, two strains (e.g. A and B) that gave a w reaction were mated and their offspring paired with each parent. Two F_1 isolates that gave the weakest v-c reactions with one or other parent (A or B) were then mated to that parent (giving two separate backcross lines) producing the F_2 generation. Both F_2 lines were paired with A and B. The F_2 isolates giving the weakest v-c reaction were selected as the parents for the F_3 generation. The procedure was repeated for the F_3 generation as it was found that at this stage, after four backcrosses, vegetative incompatibility had been eliminated in both lines (i.e. the F_4 progeny were compatible with A and B). Assuming random assortment of v-c genes this result suggested that at least three loci are involved in vegetative incompatibility even if one one v-c locus was eliminated at each generation. As there was regular segregation (1 : 1) for mating type in all four backcross generations this indicated that the v-c loci are functionally independent of the mating type locus (Brasier, 1984).

The nature of the reaction zone produced between vegetatively (somatically) incompatible groups is variable, both within species, as has been shown by O. ulmi above, and between species. Variation mainly occurs in zone width, intensity and colour of pigment produced and development of aerial mycelium on either side or within the interaction zone (Rayner et al., 1984). The aerial mycelium in some species may be temporarily heterokaryotic, for

example the dense white mycelial ridge that characteristically developed in interaction zones between paired sibs of H. serpens broke down on subculture into one or other of the original homokaryotic types. A less dramatic, but nevertheless similar phenomenon occurred in sib-pairings of D. concentrica (Dowson, 1982) and this may be analogous to heterokaryotic tufts produced within interaction zones between different genotypes of Pyricularia oryzae. In this fungus heterokaryosis was demonstrated if different monoconidial isolates from hyphal tip cultures were representative of both "parental" types and/or if new colony morphologies were recovered (Fatemi and Nelson, 1978).

In natural ascomycete populations stable heterokaryons are probably unusual because, as discussed above, somatic incompatibility mechanisms restrict their formation. Occasionally, however they do occur as was demonstrated by a survey of 63 field isolates of C. parasitica in which one was found to be heterokaryotic. This heterokaryon was considered to be ecologically viable as its genetic status did not appear to affect its pathogenicity (c.f parental isolates) nor its progeny from selfing or crossing. It was suggested that the heterokaryon had formed when young germlings fused at a stage before the self-non-self recognition (somatic incompatibility) mechanism had fully developed (Anagnostakis, 1981).

Somatic incompatibility may not only play a role in natural populations in preventing heterokaryon formation and so delimiting

individual genotypes, but it also restricts transmission of cytoplasm, that is cytoplasmic factors, between strains. Cytoplasmic transfer in C. parasitica has been found to occur rapidly between v-c groups that form weak barrages (areas of dead cells with no covering of aerial mycelium) when their mycelia meet on agar media, but occurs infrequently, or not at all, between groups that form strong barrages (Anagnostakis, 1983). This restriction of cytoplasmic transfer in C. parasitica is potentially important for the control of chestnut blight. Certain non-pathogenic strains that are capable of transferring, via hyphal fusions, cytoplasmic determinants could be used to overcome the pathogenicity of pathogenic strains. The cytoplasmic determinants are on, or associated with, double-stranded RNA molecules and are referred to as the H-factor (Anagnostakis, 1984a). A cytoplasmically transmissible element, the d-factor, in certain isolates of the aggressive strain of O. ulmi may be similar to the H-factor of Cryphonectria. Isolates that carry the d-factor (d-infected isolates) grow weakly and have reduced reproductive fitness. Further, this condition can be transferred to vigorous isolates via hyphal fusions which allow d-factor transmission. The comparison with suppression of pathogenicity in the chestnut blight fungus raises the possibility of the use of d-factors in the control of Dutch elm disease, for example by introducing d-infected isolates into elm beetle breeding galleries, in order to infect healthy O. ulmi. The system of vegetative incompatibility in O. ulmi, however, would probably impede the spread of d-factors in nature (Brasier,

1983) as it does with regard to the H-factor in the North American population of C. parasitica (Anagnostakis, 1984b).

Another phenomenon associated with vegetative compatibility in O. ulmi referred to as the "penetration effect", is also potentially ecologically significant. It appeared to be brought about mainly in w and occasionally in n type v-c reactions by hyphal interdigitation of two adjacent mycelia. It was expressed after a period of up to 40 days as lines of synnemata, belonging to the opposing isolate, along either side of the interaction zone. Penetration was observed to be uni- or bi-directional, that is into one or both isolates and the extent of penetration varied from one w reaction to another, so that isolates could be ranked according to their penetrating ability. This varied between members of a single v-c group, suggesting that penetrating ability is influenced or controlled by some factor other than v-c genes. Penetrating ability also seemed to be influenced by mycelial vigour, although it was not clear how. A further feature of the penetration effect was perithecial production, associated with the interaction zone, between isolates that were of opposite mating type. The extent and direction (uni- or bi-directional) of perithecial formation followed that of synnematal formation so that the number of perithecia produced was greatest in w type reactions, decreased in n and l types, until in lg and c type reactions perithecia were confined to the area of immediate mycelial confrontation (Brasier, 1984).

In elm bark, O. ulmi exists as a mosaic of different v-c groups, vegetative compatibility maintaining the "territory" (domain) of each genotype. Although the role of the penetration effect is not clear, it is potentially significant as a way by which one genotype can invade another's territory. If it could be demonstrated that for example a genotype of the non-aggressive strain is able to penetrate and invade the territory of a genotype of the aggressive strain, the penetration effect, like the d-factor, may be of potential use in biological control of Dutch elm disease (Brasier, 1984).

Studies of pathogen populations and a knowledge of the intra-specific recognition systems that operate within them, are obviously important as aids to understanding disease aetiology and control. Similar investigations into species that are not necessarily regarded as pathogens, can equally reveal valuable information regarding mating systems, the relative importance of sexual and asexual modes of reproduction and dissemination and ecological strategies.

In a preliminary study of the Xylariaceae by Dowson (1982), Xylaria hypoxylon and H. serpens were found to exist in wood as numerous discrete genotypes occupying small clearly demarcated domains, while D. concentrica and H. nummularium seemed to occupy large volumes of wood in attached branches or standing trunks. It was concluded that these variations in colonization and effective outgrowth were reflections of the differences between the species

in their host preference and colonization strategies. Xylaria hypoxylon and H. serpens occur on stumps and logs of any deciduous tree whereas D. concentrica and H. nummularium selectively grow on Fraxinus and Fagus respectively and colonize standing trees. In all the species, single ascospore isolates that were sib and non-sib related were all somatically incompatible indicating that somatic incompatibility is under polygenic heterogenic control. The significance of the possible temporary heterokaryosis occurring between sib-pairings of H. serpens and D. concentrica (discussed above) was not explained on the grounds of insufficient evidence, but obviously required further investigation.

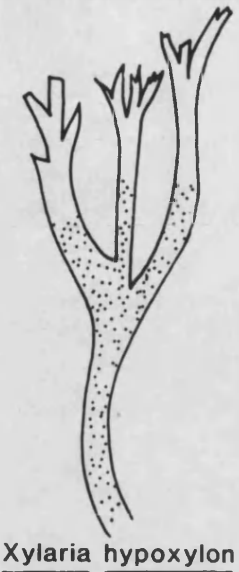
1.2 Aims of the Present Investigation

The aims of the present investigation were to provide information about a variety of xylariaceous fungi with respect to the distribution of individual genotypes in natural populations, the genetically-based variation in their mycelial characteristics, their intraspecific and interspecific recognition reactions and the possible occurrence of heterokaryosis and vegetative mycelial transitions between morphologically different forms. It was hoped that the results would elucidate stages in the life cycle and explain patterns of establishment and development in natural populations. Further they may help to explain the marked host-preferences demonstrated by certain wood-decaying members of the group towards particular tree species.

Table 1.1. Host preference and geographic distribution of some Hypoxylon species. Compiled from information by Miller (1961).

	Species	Host	Distribution
Section Hypoxylon	Hypoxylon fragiforme	Fagus. Occasionally other woody plants.	Northern Temperate Zone.
	Hypoxylon fuscum	Dead limbs of Betulaceae (chiefly Alnus, Betula and Corylus).	Northern Temperate Zone.
	Hypoxylon rubiginosum	Almost all woody dicotyledonous plants especially Acer and Fraxinus. Also the Bambuseae (monocotyledonous) and occasionally gymnosperm wood.	Worldwide i.e. not limited by thermal zones.
Section Papillata sub-section Papillata	Hypoxylon multiforme	Many woody plants, chiefly Betula.	Northern Temperate Zone, not the tropics except at very high altitude and not Southern Temperate Zone.
Section Papillata Sub-section Primo-cinerea	Hypoxylon mammatum	Acer, Alnus, Betula, Carpinus, Fagus, Picea, Populus, Pyrus, Salix, Sorbus, Ulmus.	Northern hemisphere temperate region including North America and Europe.
	Hypoxylon serpens	Many kinds of wood usually old logs and stumps.	Cosmopolitan.
Section Annulata	Hypoxylon truncatum	Many kinds of dicotyledonous trees. In United States chiefly Quercus.	Tropics - especially tropical Africa, Australia, South America, and Asia and southern United States.
Section Applanata	Hypoxylon nummularium	Deciduous wood, chiefly Fagus species.	British Isles and Europe.

Figure 1.1.Examples of stromatal form in the Xylariaceae (adapted from Corner (1968) and Rogers (1979)).



Xylaria hypoxylon

Xylaria polymorpha

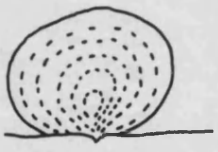
A. More less upright or branched.

for example Xylaria

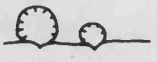
Poronia

Podosordaria

Camillea



Daldinia concentrica



Hypoxylon fuscum

B. Semi-globose or hemispherical.

for example Daldinia

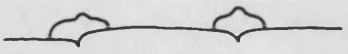
Hypoxylon

Ustulina deusta

C. Resupinate crusts.

for example Ustulina

Hypoxylon



Rosellinia spp.

D. Individual perithecia.

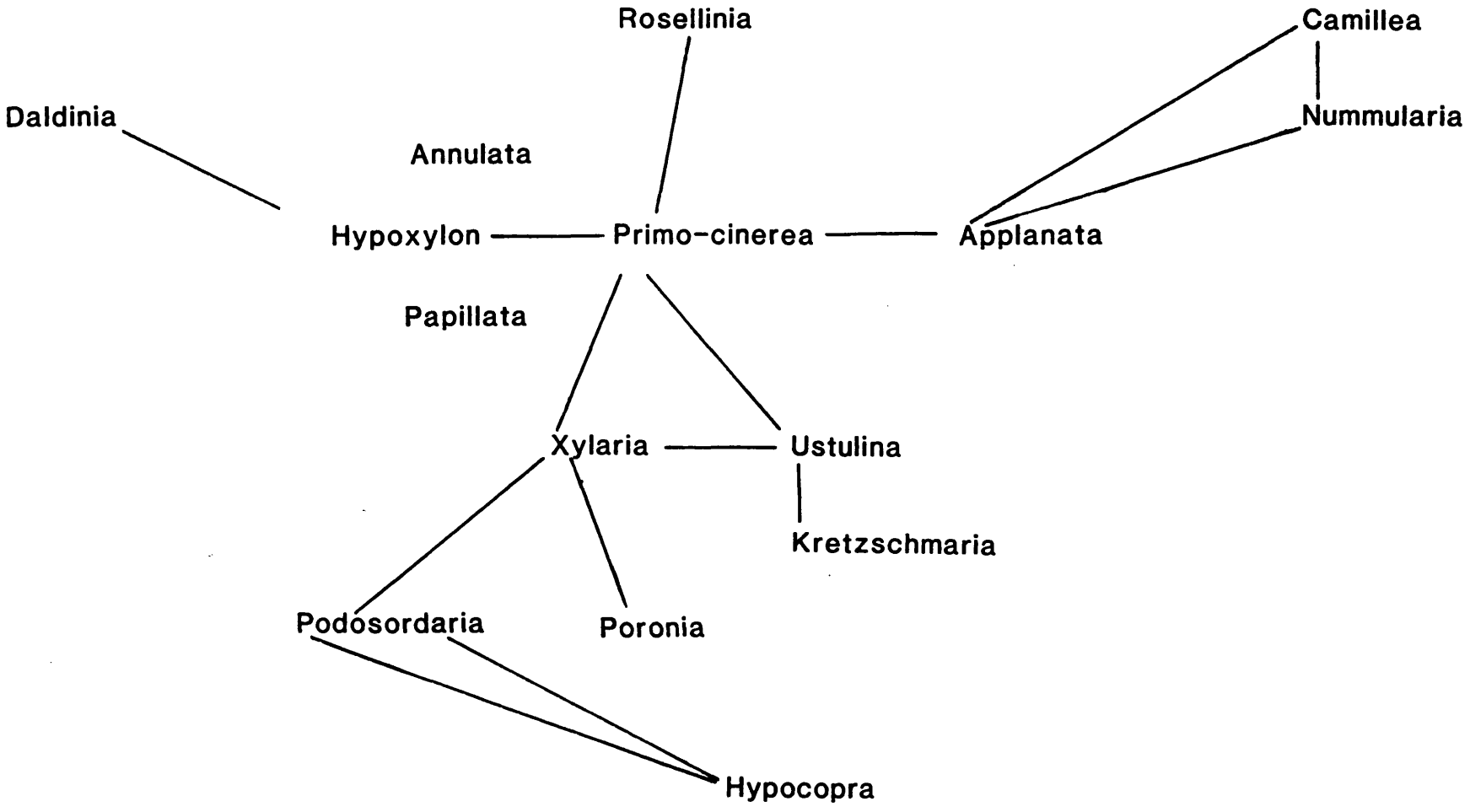
for example Rosellinia

Hypoxylon

There is a progressive loss of sterile tissue from Ato D.

The illustrations are not drawn to scale.

Figure 1.2. Relationships between the genera comprising the Xylariaceae (adapted from Whalley and Edwards (1987)).



CHAPTER 2

GENERAL MATERIALS AND METHODS

The following materials and methods were used throughout the study and are described here to avoid repetition. More specific procedures will be described later in the relevant section.

2.1 Culturing procedures

i. Media

Media suitable to support the growth of filamentous fungal colonies were prepared prior to collection of field samples. Unless otherwise specified the ingredients were dissolved, autoclaved at 121°C for 15 min and dispensed in 20 ml amounts into 9 cm plastic Petri dishes. The agar used throughout was lab M agar No. 2 (code MC6).

(a) Malt extract agar (2% w/v) (MA)

20 g malt extract (Spray Malt A. Munton and Fison Ltd., Stowmarket, Suffolk)

20 g agar

1 ℓ distilled water

When required to suppress bacterial growth, 0.01% (w/v) Novobiocin (Sigma Chemicals Ltd., Poole, Dorset) was added prior to autoclaving. MA plus 0.01% Novobiocin is abbreviated to MAN.

(b) Czapek Dox plus 2% (w/v) starch (CDS)

33.4 g Czapek Dox Liquid Medium (Oxoid CM95)

20 g starch (soluble AnalaR, BDH Chemicals Ltd., Poole, Dorset)

20 g agar

1 ℓ distilled water

This medium was autoclaved at 115°C for 20 min.

ii. Cultural conditions and maintenance of cultures

Except where otherwise stated, cultures were grown on MA Petri dish plates and incubated facing upwards at 20°C in darkness. They were maintained by taking 6 mm diameter plugs of mycelium plus agar from the edge of actively growing colonies and transferring these inocula to fresh MA. Cultures were stored under sterile liquid paraffin (S.G. 0.86 - 0.89, water white. Fisons Scientific Apparatus Ltd., Loughborough, Leicestershire) on 2% MA slopes in bijoux bottles. These were kept at 4°C. Aseptic technique was used throughout.

2.2 Sampling sites

Samples of decaying wood and perithecial stromata of various xylariaceous species were collected from sites in Great Britain and occasionally from abroad (Table 2.1).

2.3 Isolation procedures

i. From wood

Fragments, approximately 10 mm^3, cut from wood which had been surface-sterilized in 5% domestic bleach (Domestos, Lever Brothers Ltd.) for 5 min, were transferred to MAN. Plates were incubated

until sufficient mycelium had grown out to enable subcultures to be made.

ii. From stromata

Fruit bodies were wiped with cotton wool soaked in 70% ethanol and then broken open to expose fresh tissue from which small pieces, approximately 10 mm^3 , were excised and placed on MAN, incubated and subcultured as for wood isolates.

iii. From spores

Single ascospore cultures were obtained from asci excised from individual perithecia, or in D. concentrica by using the spore-tendrils or cirri which occur when the normal violent ascus discharge mechanism fails (Ingold, 1971). The asci (or in D. concentrica a single cirrus) were gently transferred, using a sterile needle, to a drop of sterile distilled water. After mixing, the drop was spread across a series of MAN Petri dish plates which were then incubated at 15°C in darkness. Single well-separated germlings were marked using a flame-sterilized dummy objective and transferred to fresh MA using a tungsten needle.

Single conidial isolates were obtained in the same way as described above, the initial spore suspension being prepared by transfer of spores from conidial cultures to, and agitation in, a drop of sterile distilled water.

2.4 Cultural characteristics

The texture and colour of mycelia were described using the terminologies of Stalpers (1978) and Rayner (1970) respectively. Where a single colour was intermediate between two coloured squares (Ridgway chips) of Rayner's chart, for example between honey and isabelline, this was written as "honey/isabelline". Where however a range of colours from honey through to isabelline was apparent, this was recorded as "honey to isabelline".

Radial extension rates were measured by marking the position of the colony margin, at 24 h intervals, along two diameters drawn at right angles on three replicate plates. These contained 2% MA (25 ml in 9 cm Petri dishes, or for D. concentrica 75 ml in 14 cm Petri dishes) and were incubated in darkness at 20°C. The first measurement was made following a lag period of 72 h.

2.5 Experimental pairings

Experimental pairings were made by placing 6 mm diameter discs of inoculum, cut from the margin of actively growing colonies, up to 1 cm apart near the centre of plates. Plates were incubated for up to 63 d and examined at regular intervals.

2.6 Isolation of single hyphal tips

A method for single hyphal tip isolation devised by Butler (1984) was followed. This required that the mycelium from which the tip was to be taken should be growing on a disc of sterile cellophane (Grade 325 P 80 mm, Cannings Parry Packaging Ltd.)

overlying agar media. The cellophane discs were sterilized by autoclaving in distilled water at 115°C for 20 min. Once the colony was established on the cellophane a hypha was selected using a binocular dissecting microscope and adjacent hyphae gently swept away with a sterile mounted needle. A square of cellophane supporting the selected hyphal tip was then excised and transferred to the centre of a fresh MA plate. Later, when the hypha had grown onto the agar surface, the cellophane square was removed.

Where this method was employed in the study of colony ontogony (Chapter 3, Section 3.2, ii,(a)), the cellophane overlaid MA and the cultural characteristics of colonies arising from single hyphal tips were described. Where hyphal tip isolation was used for testing for heterokaryosis in the region of aerial mycelium (am), characteristically produced between interacting strains (Chapter 4, Section 4.3, iii), cellophane overlaid CDS. Small pieces of mycelium taken from the am and transferred to the cellophane and CDS, produced colonies after 2-3 d incubation in the dark, that were sufficiently diffuse at their margin for easy excision of single tips. When the colony arising from the excised tip was well established, inocula of the two strains originally paired were placed on either side of it (or subcultures of it) on the same diameter at the edge of the plate, and the resulting interactions between the three colonies recorded.

Table 2.1. Type of wood bearing xylariaceous perithecial stroma/stromata and number of logs sampled at different sites.

Species	Site	Site code	National Grid Reference	Type of wood	Number of samples
Daldinia concentrica	Ashclyst Forest, Devon	A3	SX 9999	ash(*Fraxinus excelsior*)	1
	Bath University Campus, Avon	B1	ST 7764	ash	2
	Bathwick Woods, Avon	B2	ST 7764	ash	8
	Browns Folly Nature Reserve, Avon	B5	ST 7964	ash	1
	Clovelly, Devon	C3	SS 3225	ash	1
	Durham City, Co. Durham	D3	NZ 284423	ash	1
	Friary Woods, Avon	F	ST 7858	ash	4
				beech (*Fagus sylvatica*)	1
	Gainford, Co. Durham	G	NZ 185166	ash	1
	Long Ashton Park, Avon	L2	ST 5572	beech	1
	Nayland, Suffolk	N1	TL 3497	ash	1
	North Newbald, Humberside	N2	SE 9137	ash	1

Table 2.1. (continued).

Species	Site	Site code	National Grid Reference	Type of wood	Number of samples
Hypoxylon fragiforme	Ashclyst Forest, Devon	A3	SX 9999	beech	2
	Colerne Woods, Wiltshire	C4	ST 798725	beech	1
	Hafod Wood, Erddig Estate, North Wales	H	SJ 6321	beech	1
	Leigh Woods, Avon	L1	ST 556732	beech	1
	Venbridge Wood, Devon	V	SX 7794	beech	1
	Waterly Bottom, Gloucestershire	W1	ST 7695	beech	1
Hypoxylon fuscum	Castle Combe, Wiltshire	C1	ST 8277	hazel (Corylus avellana)	2
	Clifford Bridge Woods, Devon	C2	SX 7889	hazel	1
	Friary Woods, Avon	F	ST 7858	hazel	5
	Manor Wood, Bristol, Avon	M	ST 573692	hazel	4
	Sutton Farm, Bow, Devon	S3	SS 723025	hazel	2
	Venbridge Wood, Devon	V	SX 7794	birch (Betula sp.)	1

Table 2.1. (continued).

Species	Site	Site code	National Grid Reference	Type of wood	Number of samples
Hypoxylon mammatum	Ddol Uchaf, Raeme, North Wales	D1	SJ 1471	willow (*Salix* sp.)	2
	Onondaga County, United States of America	O	-	trembling aspen (*Populus tremuloides*)	1
Hypoxylon multiforme	Arlington Court, Devon	A2	SS 6040	silver birch (*Betula pendula*)	1
	Ashclyst Forest, Devon	A3	SX 9999	silver birch	2
	Clifford Bridge Woods, Devon	C2	SX 7889	hazel	1
	Conkwell Woods, Wiltshire	C5	ST 786625	common alder (*Alnus glutinosa*)	4
	Delamere Forest, Cheshire	D2	SJ 5471	beech	1
	Friary Woods, Avon	F	ST 7858	silver birch hazel	3 1
	Leigh Woods, Avon	L1	ST 556732	silver birch	1
	Longleat, Wiltshire	L3	ST 8443	silver birch	1
	Savernake Forest, Wiltshire	S1	SU 2267	silver birch	5
	Steps Bridge, Devon	S2	SX 8088	silver birch	1

Table 2.1. (continued).

Species	Site	Site code	National Grid Reference	Type of wood	Number of samples
Hypoxylon multiforme	Uppsala, Sweden	U	-	silver birch	3
	Venbridge Wood, Devon	V	SX 7794	silver birch	4
	Wetmoor, Chipping Sodbury, Avon	W2	ST 7487	silver birch	1
Hypoxylon nummularium	Clifford Bridge Woods, Devon	C22	SX 7889	beech	1
	Colerne Woods, Wiltshire	C4	ST 798725	beech	1
	Friary Woods, Avon	F	ST 7858	beech	3
	Venbridge Wood, Devon	V	SX 7794	beech	1
"*Hypoxylon purpureum*"	Bath University Campus, Avon	B1	ST 7764	beech	1
	Browns Folly Nature Reserve, Avon	B5	ST 7966	beech	1
	Friary Woods, Avon	F	ST 7858	beech	1
	Hafod Wood, Erddig Estate, North Wales	H	SJ 6321	beech	1

Table 2.1. (continued).

Species	Site	Site code	National Grid Reference	Type of wood	Number of samples
Hypoxylon rubiginosum	Ashclyst Forest, Devon	A3	SX 9999	ash	6
	Browns Folly Nature Reserve, Avon	B5	ST 7966	ash	1
	Friary Woods, Avon	F	ST 7858	ash	1
	Uppsala, Sweden	U	-	decorticated wood	1
	Venbridge Wood, Devon	V	SX 7794	ash	1
Hypoxylon serpens	Bigwood, Erddig Estate, North Wales	B3	SJ 3248	decorticated wood	1
	Britannia Wood, Bangor, Gwynedd	B4	SH 5772	oak (*Quercus* sp.)	1
	Delamere Forest, Cheshire	D2	SJ 5471	beech	1
	Friary Woods, Avon	F	ST 7858	decorticated wood	2
	Venbridge Wood, Devon	V	SX 7794	decorticated wood	1
Rosellinia desmazieresii	Ainsdale Sand Dunes Nature Reserve, Lancashire	A1	SD 2912	creeping willow (*Salix repens*)	9
Rosellinia mammiformis	Venbridge Wood, Devon	V	SX 7794	ivy (*Hedera helix*)	1

CHAPTER 3

COLONY ONTOGENY

3.1 Introduction

During natural colonization processes the fungal thallus is often sequentially exposed to a wide variety of often contradictory environmental conditions in space and time, from the moment of spore germination through exploration, resource capture and competition with other organisms of the same and different species, to reproduction (Cooke and Rayner, 1984). It is not fully understood how a thallus copes with these changing selection pressures, but it has been proposed that the mycelium of many higher fungi (Ascomycotina and Basidiomycotina) is able to counter them by switching between a variety of morphologically and functionally distinctive developmental states (Rayner, Watling and Frankland, 1985; Rayner and Coates, 1987; Rayner, Boddy and Dowson, 1987). Such states may, following Gregory (1984), usefully be termed "modes" and transitions between different mycelial modes may include determinate (unicellular)-indeterminate (filamentous) transitions, alterations in internode length and branch angle of hyphae, aerial versus appressed or submerged growth, compacted versus diffuse morphogenesis and "juvenility" versus "senescence" (Rayner and Coates, 1987).

The fungal thallus presumably contains within its developmental programming certain basic morphogenetic options, and expression of different mycelial modes may be regulated genetically by a series

of superimposable switch mechanisms which enable change-overs between different developmental pathways. In turn these developmental switches may be cued by a wide variety of endogenous and exogenous stimuli, so that for example a particular environmental parameter may act as a cue for a switch to a particular developmental pathway, resulting in alteration from one mycelial mode to another more suited to cope with current conditions (Rayner and Coates, 1987). Even under homogeneous pure culture conditions used in the laboratory, in which environmental variables tend to be standardized - a situation far removed from the heterogeneity which characteristically occurs in nature - the same mycelial genotype can adopt distinctive patterns of development. Some of this variability in colony ontogeny may arise from random switching under non-selective conditions. It is important to establish the extent to which this may apply in order to understand the developmental switching that may be triggered by exogenous factors including mycelial interactions between different fungal strains (Rayner and Coates, 1987) which are to be described later (Chapter 4).

A knowledge of the cultural characters of unpaired mycelia is also useful because mycelial isolates obtained from xylariaceous fungi may be difficult to identify in the absence of a stroma; most taxonomic keys and descriptions are based upon characters of the teleomorph which is rarely produced in culture and although descriptions of the anamorph can indicate the correct genus, further identification is often not easy (Petrini and Petrini,

1985). Comparison of cultural characters of unidentified isolates with accurate descriptions of those of single ascospore isolates of known species can aid identification, but to date the cultural characters of only a limited number of xylariaceous species have been described (Greenhalgh and Chesters, 1968; Jong and Rogers, 1972; Petrini and Petrini, 1985).

The aim of this chapter is to describe the cultural characters of xylariaceous species (single ascospore and wood isolates) under homogeneous environmental conditions, and to study the relative importance of endogenous (genetic) and exogenous (environmental) controls in the production of morphologically distinct mycelial types in those species that exhibit them. A brief examination of the development of pigmentation in Hypoxylon fragiforme colonies will be described, as well as a preliminary screening of xylariaceous species for extracellular enzyme (phenol oxidase and peroxidase) production. These are key enzymes associated with lignin breakdown and there is evidence that they may be partitioned between two developmental states of the mycelium of Phellinus tremulae, a basidiomycete causing heart rot of aspen (Populus tremula). A slow growing pigmented colony form possesses laccase and tyrosinase activity, whilst a fast growing, aerial, non-pigmented form possesses only peroxidase activity (Hiorth, 1965). Similarly, the mycelium of the wood-decaying basidiomycete Hymenochaete corrugata occurs in two developmental states, an appressed yellow-brown pigmented form and a white aerial form (Sharland, Burton and Rayner, 1986). The former appears to be

associated with more decayed areas of wood and possesses tyrosinase and laccase activities whilst the latter possesses no such activity (Sharland and Rayner, unpublished).

Throughout this chapter abbreviations and strain codes specific to this investigation have been used. These are listed in full in Appendix II.

3.2 Materials and Methods

i. Cultural characters

The cultural characters of strains inoculated onto 2% MA and incubated in darkness were described and radial extension rates of Daldinia concentrica and Hypoxylon nummularium were measured using methods explained in Chapter 2 (Section 2.4). The radial extension rates of other species were estimated from the colony size after 7 d.

ii. Morphologically distinct mycelial types in some species

Morphologically distinct mycelial types within single colonies or between colonies of some species were examined using one or more of the methods below.

(a) The cultural characters of colonies arising from excised single hyphal tips (using the procedure described in Chapter 2, Section 2.6) were recorded. In "Hypoxylon purpureum" single lateral branches from dull green mycelium were isolated with

the use of a Singer micromanipulator (Singer Instrument Co. Ltd., Reading, England).

For Hypoxylon fragiforme radial extension rates of hyphal tip colonies were measured using the method explained in Chapter 2 (Section 2.4). The extension rate was calculated as the slope of linear regression of radial extension against time (mmd^{-1}).

(b) The following features were measured for the fastest-extending (leader) hypha in each 1000 µm of colony margin of cultures grown over cellophane (Grade 325 P 80 mm, Cannings Parry Packaging Ltd.) and 2% MA and examined at 1 h intervals:

(1) the length from tip to first branch of the leader hypha;

(2) the length of the first branch;

(3) the extension rate ($\mu m\ h^{-1}$) of the leader hypha and first branch;

(4) the angle between the first branch and the parent axis;

(5) for Hypoxylon serpens only, the internode length between first and second, and second and third branches, the length of second and third branches and the angle between second/third branches and the parent axis.

(c) The colony form of strains (two replicates each) kept in darkness was compared with that of the same strains exposed to white fluorescent light (12 h in every 24 h or continuous). Incubation under each regime was at 20°C.

(d) The cultural characters of a minimum of 20 single conidium isolates prepared from different parent colonies were recorded. The procedure for obtaining single conidium isolates is described in Chapter 2 (Section 2.3, iii).

(e) The cultural characters of colonies (two replicates per strain) produced in darkness at 5, 15, 20, 25, 30 and 37°C were recorded.

In methods (a) to (e) colony or zone diameters were measured along two perpendicular lines on a minimum of two replicate Petri dish plates. Unless otherwise stated the culture medium was 2% MA and incubation was at 20°C in darkness.

The species examined and the methods used were H. fragiforme (a), (b), Hypoxylon multiforme (a), (b), "H. purpureum" (a), (c), (d), (e) and H. serpens (a), (b), (c) and (d).

iii. Development of pigmentation in Hypoxylon fragiforme colonies

The development of pigmentation in colonies of H. fragiforme was studied using two methods:

(a) Pigmentation of five strains (two replicates for each treatment) kept in darkness was compared with that of the same strains exposed to light (12 h in every 24 h and continuous light);

and

(b) The development of pigmentation in colonies of a strain derived from a single ascospore monitored at 0, 2, 9, 16 and 35 d following exposure to various periods (0, 3, 6, 9 and 12 h) of light. Colonies were incubated in darkness for 5 d prior to light exposure to ensure that they were well established.

In (a) and (b) inocula were taken from 7 d old, non-pigmented colonies and pigmentation was assessed by measuring the extent of the pigmented area along two diameters drawn at right angles on two replicate 9 cm Petri dishes of 25 ml 2% MA. Pigmentation was expressed as a percentage, i.e.

$$\frac{\text{diameter of pigmented area}}{\text{diameter of colony}} \times 100$$

Incubation was at 20°C. Except for the two light treatments in (a), cultures were wrapped in silver foil to ensure the exclusion of light and all measurements were made under a Kodak safelight (filter OA CAT 1521491).

iv. Phenol oxidase and peroxidase activity

Strains were tested for phenol oxidase and peroxidase activity by adding phenols and pyrogallol reagent plus hydrogen peroxide respectively to 6 mm diameter wells cut with a cork borer into the mycelium and agar at the edge of colonies, and observing the reaction. The phenols (0.01% w/v solutions) used were salicylic acid, quinol, catechol, guaiacol (all from BDH Chemicals Ltd., Poole, Dorset) and ferulic acid (Sigma Chemicals Ltd., Poole,

Dorset) to test for laccase activity and dihydroxyphenylalanine (Sigma Chemicals Ltd.) to test for tyrosinase. The development of colour in the agar around the wells was recorded after 1, 3, 6 and 18 h. For the test for peroxidase activity 0.5 ml of 1% (v/v) hydrogen peroxide (BDH Chemicals Ltd.) was added and mixed with 1.5 ml pyrogallol reagent in each well. The pyrogall reagent was freshly prepared : 10 ml 0.5 M pyrogallol (BDH Chemicals Ltd.), 12.5 ml 0.66 M phosphate buffer (K_2HPO_4 and KH_2PO_4, Fisons Scientific Apparatus Ltd., Loughborough, Leicestershire) pH 6.0 made up to 100 ml with water. Peroxidase activity was assessed on an arbitrary scale (NR = no reaction; + - gas production around well periphery only; ++ - gas production over whole surface of well; +++ - gas production up to 1 mm above surface of well) after 30 min.

3.3 Results

i. Cultural characters

The cultural characters and growth rates of strains on 2% MA are shown in Table 3.1 and Figure 3.1, (A) - (K).

In most species there was little variation in cultural appearance so that strains, whether from within or between progeny sets (a progeny set being monospore isolates from a single perithecium) or from geographically separate wood samples, produced similar colonies. A few species however produced different types of morphologically distinct mycelia ("mycelial types") within or between colonies. For example isolates of H. serpens produced

colonies of "grey conidial" or "white silky" mycelial types. These two were not recorded within the same progeny set and were only found together once, on a log sample following incubation (Figure 3.2). By contrast, in the single progeny set examined of Rosellinia mammiformis, colonies varied from "velvety slow" to "cottony fast" mycelia including intermediate types. A proportion of isolations from wood and single ascospores of Hypoxylon fuscum and H. multiforme resulted in colonies displaying a sector or sectors of a mycelial type distinctive from that 'typical' (described in Table 3.1) for the species. In H. fuscum such strains produced colonies of an irregular mycelial mat of 'typical' buff or honey mycelial fans emerging from slow dense, dull green or isabelline pigmented mycelium (Figure 3.3). The H. multiforme strains developed one or more fan-shaped sectors where growth of the colony margin was restricted (Figure 3.4). A mycelial type where growth (linear and aerial) appeared to be restricted (R type) compared to the 'typical' mycelial type in which growth was unrestricted (U type) was also a feature of some H. fragiforme strains, in which R mycelium was appressed to the agar surface with sparse aerial hyphae and was frequently associated with cinnamon to sepia or dark bluish green pigment in the medium. Some colonies were completely composed of R type mycelium and developed sparsely from the inoculum, while others grew out normally (producing U type mycelia) but at a later stage produced an R type sector or sectors (Figure 3.5, A and B).

The mycelial types described above for H. fragiforme, H. multiforme and H. serpens, as well as new ones which developed after subculturing some isolates of "H. purpureum" and H. serpens, were examined in more detail (see below).

After 21 d incubation all species except Rosellinia desmazieresii and Hypoxylon rubiginosum produced conidia. In most species these were formed on and around the inoculum at first and then spreading outwards, sometimes over most of the colony, for example in some Daldinia concentrica isolates. In other species, such as H. fragiforme, conidial areas were restricted to irregular shaped patches. All the spores were borne on nodulose conidiophores except for H. serpens where conidiophores were geniculate. None of the species produced any structure resembling a perithecium or stroma.

ii. Morphologically distinct mycelial types in some species

(a) Restricted (R) and unrestricted (U) mycelial types in Hypoxylon fragiforme

A comparison of R and U mycelial types in six strains derived from single ascospore (as) and four strains derived from wood (w) isolates (selected because they produced both kinds of mycelia) showed that within any one strain there was a difference in radial extension rates (mmd^{-1}) between the two mycelial types (Figure 3.6). Between strains however there was overlap in the extension rates of R and U, so that the extension rate of R in one strain was the same as that of U in another strain (e.g. extension rate class

0.35 - 0.399 mmd^{-1} includes R type of w1 and U type of as1, 2, 3, 4, 5 and 6). Similarly the mean values for extension rates of R and U varied considerably between strains from classes 0.15 - 0.199 mmd^{-1} (as1) to 0.3 - 0.349 mmd^{-1} (w2) in the former and from 0.4 - 0.449 mmd^{-1} (as1, 2, 3 and 5) to 0.5 - 0.549 mmd^{-1} (w1,2, 3 and 4) in the latter. Of the ten strains selected, those from ascospores had slower radial extension rates than those from wood and two of the latter failed to produce any R type mycelia.

Microscopic examination of the two mycelial types (Figure 3.7 and Table 3.2) revealed that the length of leader hyphae from tip to first branch was always shorter in the R (114 - 295 µm) than in the U (492 - 729 µm). At the same time, within any one strain the extension rate of leader hyphae was slower in R than in U and, excluding isolate w2, varied from 15 to 29 µm h^{-1} in R and from 52 to 159 µmh^{-1} in U. The extension rate for U leader hyphae in w2 was 22 µm h^{-1} (+/- 12 µm h^{-1}*) which was faster than the rate for its R leader hyphae (10 µm h^{-1} +/- 5 µm h^{-1}*) but was equivalent to the R rates of other strains. The angle between the first branch and parent axis, and the first branch length, did not differ greatly between the two types (51-74° and 32-63 µm for R and 61-85° and 21-65 µm for U) although within each strain the first branch extension rate relative to that of the parent hypha was always faster in R than in U. Between strains there was overlap in these values which were 21% to 118% for R and 3% to 82% for U.

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* +/- x 95% confidence intervals

Study of the stability of R and U mycelial types showed that colonies arising from excised hyphal tips were not always of the same type (R and U) as the mycelium which the hyphal tip came from (Table 3.3). In only two cases (as1, R with U sector and as3, R) were all ten hyphal tip colonies of the same mycelial type as the original hyphae. Usually only a proportion of the colonies remained true to the original type. The remainder either grew as the alternate mycelial type and/or grew as the original type but produced a sector or sectors of the alternate one. In two instances (as1, U with R sector and as3, R with U sector) all ten colonies arising from tips of one type grew in the other mode. However, in both cases, the original colonies from which the tips were taken, showed both R and U sectors. In contrast to this, seven out of ten colonies of w2 were U and yet the tips they arose from came from a totally R type colony.

(b) Restricted (R) and unrestricted (U) mycelial types in Hypoxylon multiforme

The same terms, restricted (R) and unrestricted (U), were used to describe mycelial types in H. multiforme and H. fragiforme, as in both species the R mycelium appeared to have a restricted linear extension rate and was appressed to the agar surface with little aerial growth, in contrast to the 'typical' U mycelium. There were however major differences in the R mycelial type of the two species: (1) in H. multiforme the R mycelial type was usually confined to small (approximately 40°-90° and < 20 mm long) sectors formed at the colony margin of freshly prepared single ascospore

isolates. Routine subcultures of these isolates rarely displayed R type sectors, probably because inocula were taken from U type areas. Unlike H. fragiforme, colonies were never completely R, except those arising from single hyphal tips taken from R mycelium (see below). (2) Pigment was always associated with R mycelium in H. fragiforme, but in H. multiforme only sometimes was a pale vinaceous to livid vinaceous pigment apparent along the edges of R sectors.

As R sectors in H. multiforme were small it was difficult to collect data on linear extension rates, although from the appearance of colonies R mycelium obviously extended more slowly than U mycelium. This observation was confirmed by a comparison of the extension rates of R and U leader hyphae (Table 3.4) (which showed that within any one isolate R hyphae extended more slowly than U hyphae). Between isolates however there was overlap in extension rates, those for R varying from 7 to 43 μmh^{-1} and those for U varying from 22 to 99 μmh^{-1}.

Comparison of leader hyphae (mean values) of R and U sectors in seven single ascospore isolates (Figure 3.8) showed that within isolates the length of the leader (from tip to first branch) was always shorter in R than in U, but there was no real difference between R and U in the angle the first branch subtends with the parent axis. First branch length was usually marginally larger in R than in U, except in isolate 2 where the first branch was much

longer in U (127 µm +/- 68 µm*) than in R (29 µm +/- 52 µm*). Between isolates however, there was considerable overlap in values of growth parameters (Table 3.4). The length of leader hyphae varied from 95 µm to 306 µm in R and from 216 µm to 545 µm in U, and first branch length varied from 27 µm to 54 µm and 25 µm to 127 µm respectively. The range of angles between the first branch and parent axis was more or less equal between the two mycelial types, standing at 30° to 67° for R and 31° to 53° for U. The first branch extension rate (3-14 $\mu m h^{-1}$ for R and 3-23 $\mu m h^{-1}$ for U) was also similar between R and U, although in most isolates it was a little faster in the latter. In contrast the first branch extension rate relative to that of the leader hypha was generally greater in R (33% - 150%) than in U (13% - 78%).

Both R and U mycelial types did not appear to be stable, as ten colonies arising from single hyphal tips taken from either an R or a U sector (Table 3.5) were only once (as5, R) all of the same mycelial type as the original hyphal tips (i.e. showing some R). Even tips isolated from U mycelia produced a proportion of colonies with some R even if this was only apparent after 7 d incubation and had altered to U by 21 d (e.g. as4, U and as2,U). In as5, U, seven out of ten hyphal tip colonies were R at 7 d and still had R sectors after 21 d incubation. The reverse situation was also observed, that is R hyphal tips giving rise to U colonies, although it only occurred at a low frequency (as1, R 3/10; as3, R 3/10; as5, R 0/10). More usually at least some R mycelium was evident from R

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* +/- x 95% confidence intervals.

hyphal tips and colonies that were R at 7 d but U by 21 d were often produced, although such colonies had not been previously recorded for H. multiforme.

(c) Concentrically zoned (CZ) and "Typical" (T) colonies of "Hypoxylon purpureum"

"Typical" (T) colonies of "H. purpureum" were composed of dull green velvety mycelium (DG) of pigmented hyphae with prolific lateral branches (Figure 3.9) surrounded by an even-edged margin (up to 2 mm wide) of white silky appressed mycelium (WA) and becoming ($\geq$ 21 d incubation) buff and conidial (BC) around the inoculum (Table 3.1 and Figure 3.1, G). Colonies reached a maximum diameter of between 50 mm and 55 mm after 28 d incubation and were often surrounded by olivaceous green or sepia pigment in the agar. A strong bitter-sweet smell, reminiscent of bitter almonds was associated with T colonies.

Most subcultures of wood and single ascospore isolates resulted in T colonies, but occasionally colonies with different cultural characteristics were produced. For example a strain derived from an ascospore isolate (H5) consistently yielded T colonies for four successive subcultures. However,on the fifth one, a concentrically zoned (CZ) colony was produced. This had a central area (35 mm diameter) of white to buff, downy to cottony textured mycelium (WDC) in which conidia/conidiophores and lateral branches were absent, surrounded by narrow (4 mm) bands of DG and WA. Even after 35 d incubation conidia/conidiophores were absent from this colony.

As a result of this alteration in colony form, single conidium isolates were prepared from other single ascospore strains in an attempt to find if the alteration was a common phenomenon.

Conidial germination and initial outgrowth was slow, but by 10 d to 14 d most single germlings had established colonies visible to the naked eye. A minimum of 20 conidial isolates of each of the ascospore isolates H8, H15 and H17 belonging to one progeny set, and 2Y19 and 2Y20 belonging to another, were prepared, of which those from H15, H17 and 2Y19 all produced T colonies, as did the majority of isolates from H8 and 2Y20. Six conidial isolates from H8 and ten from 2Y20 however, developed into CZ colonies (Figure 3.10). These were composed of bands of varying widths (2-9 mm) of different mycelial types, including DG, WA, WDC, BC and BC with brown liquid droplets (L). In some zones the mycelium was raised into mounds (here the prefix M is used; e.g. MBC). Generally the central zone around the inoculum was BC, but thereafter the order of successive mycelial types varied. After 35 d incubation the zones from the centre outwards were for example BC (9 mm), L (2 mm), WDC (3 mm), BC (7 mm), DG (7 mm) and WA (2 mm) in one isolate, whilst BC (9 mm), MBC (3 mm), DG (6 mm), ML (6 mm), BC and DG (8 mm) and WDC (8 mm) in another (the width of each zone is given in brackets). CZ colonies reached a maximum diameter of between 70 mm and 85 mm after 28 d incubation and there was no pigment in the agar.

After 45 d incubation one conidial isolate of H15, two of H17 and one of H8 which all initially produced T colonies, had developed broad ($\geq$ 10 mm) WDC bands around the margin (Figure 3.11). Subcultures from the WDC area of H15 and one isolate of H17 produced T colonies. Subcultures from the other H17 and H8 isolates resulted in CZ colonies (including WDC bands).

Ten second generation conidial isolates prepared from each of a first generation isolate of H15, H17 (T colonies) and H8 (CZ colony) all yielded T colonies. From the margin of one of these T colonies (second generation H8) a WDC mycelial fan was produced. Subcultures from this fan resulted in CZ colonies including L and WDC bands.

Colonies arising from T and CZ inocula that were experimentally paired were of the same colony type as the inoculum. That is the presence of,for example T adjacent to CZ, did not cause either to alter colony type, even when the isolates were somatically compatible (i.e. conidial isolates, one T and one CZ, from the same strain of ascospore isolate).

Altering the incubation temperature did not appear to have any effect on colony type (Table 3.6) so that colonies arising from the same inoculum were either all T or all CZ at 15, 20 and 25°C. Growth was limited to the immediate vicinity of the inoculum or did not occur at all at temperatures of 5, 30 and 37°C. Inocula from a T colony (as1) all produced T colonies, as did inocula from BC and

DG areas of two CZ colonies (C1 and C2) derived from single conidia. Inocula from WDC of a third CZ colony (C3) however all produced CZ colonies.

Similarly colonies arising from excised WDC hyphal tips from a CZ colony (Table 3.7) mostly produced CZ colonies (8/10 when CZ colony 7 d old; 6/10 when CZ colony 21 d old) while colonies arising from excised single lateral branches (from DG mycelium) of the same CZ colony, were all T type. Indeed T colonies were always produced from lateral branches of DG zones. T colonies also developed from all single WA hyphal tips (of T colonies) except for three from strain as1 which resulted in CZ colonies. This was the original strain that after repeated subculture had produced the first CZ colony to be observed.

Consistent with the above observations WA inocula from T colonies yielded T colonies in darkness, however exposure to light resulted in CZ colonies (Table 3.8). These light-induced CZ (LICZ) colonies differed from those previously recorded in two ways. Firstly many of them possessed a honey to isabelline felty textured mycelium (HF) which appeared to be intermediate between WDC and BC. Areas that were HF after 21 d incubation became BC by 35 d. Secondly all LICZ colonies had pigment in the agar, although there was considerable variation, even between replicates, as to its colour. Associated with some of them was a faint sweet smell of roses which may have been a less intense form of the more familiar bitter-almond smell. LICZ colonies from the continuous light

treatment were always considerably larger in diameter than those from the 12 h light/12 h dark and all had a central BC zone which was lacking in the latter. The largest LICZ colonies were produced by the strain derived from the ascospore isolate and the smallest by the conidial one, although this probably only indicated inherent differences between strains.

(d) White silky (ws), white felty (wf) and grey conidial (gc) mycelial types in Hypoxylon serpens

As explained above, isolates of H. serpens were either white silky (ws) or grey conidial (gc) mycelial types (Table 3.1). A third mycelial type referred to as white felty (wf) arose when ws colonies of single ascospore strains were subcultured after prolonged incubation (> 90 d). Such subcultures produced a range of colony forms which after 12 d incubation at 20°C in darkness varied from small (25 mm diameter) spherical, even-edged wf colonies, through wf (25 mm diameter) with fast extending ws mycelial fans (i.e. wf/ws colonies) (Figure 3.12, A and B) to large (77 mm diameter) ws colonies. Colonies that were wf/ws were the most common type. Except for a narrow (100 µm) margin, the surface of wf colonies was covered in geniculate conidiophores which were similar to, but less densely packed than, those on the surface of the gc colonies. Conidiophores and conidia were absent from ws colonies and fans.

A comparison of the growth characteristics of leader hyphae of ws, wf and gc mycelial types (Figure 3.13, Table 3.9) revealed that

wf leaders differed markedly from ws and gc ones as the first and second branches were much shorter (12 µm +/- 4 µm*, 17 µm +/- 6 µm* for wf; 37 µm +/- 2 µm*, 117 µm +/- 6 µm* for ws and 69 µm +/- 22 µm*, 43 µm +/- 22 µm* for gc respectively). Branch length of ws leaders increased successively from first (37 µm +/- 2 µm*) through second (117 µm +/- 6 µm*) to third branches (200 µm +/- 21 µm*), but an irregular pattern was recorded for gc leaders. The second branch was shorter (43 µm +/- 22 µm*) than both the first (69 µm +/- 22 µm) and third branch (111 µm +/- 62 µm*).

In two features ws leaders were clearly distinguished from the other two types between which measurements overlapped. For example the length of leader from tip to first branch was longest in ws (201 µm +/- 4 µm*) but for wf (148 µm +/- 26 µm*) and gc (125 µm +/- 31 µm*) the values partly coincided. Similarly the internode length between first and second branches was longest in ws (192 µm +/- 5 µm*) and overlapped for wf (160 µm +/- 23 µm*) and gc (172 µm +/- 50 µm*). In internode length between second and third branches however there was overlap in the values obtained for all three types (ws 162 µm +/- 6 µm*; wf 147 µm +/- 41 µm* and gc 132 µm +/- 43 µm*).

The angle between a branch and the parent axis was consistent for successive branches (i.e. first, second and third branches) in ws and wf mycelial types varying from 26° (+/- 6°*) to 31°

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* +/- x 95% confidence intervals.

(+/- 7°*) in ws and 50° (+/- 10°*) to 56° (+/- 10°*) in wf. However, the branching angles for gc leaders appeared to be erratic so that it was 31° (+/- 8°*) for first, 62° (+/- 8°*) for second and 51° (+/- 13°) in third branches.

Single conidial isolates prepared from wf and gc colonies always developed mycelial morphology of the parental type. wf conidia germinated after 4 d at 20°C in darkness and over a 28 d incubation period there was little variation in the appearance of 25 isolates. The colonies were composed of a central hemispherical mound (6 mm diameter, 2 mm high) around the inoculum, of initially wf mycelium (7 d) becoming wf with smoke grey conidial patches (> 14 d) surrounded by wf (to a diameter of 32 mm +/- 6 mm*) and ws zones (to a diameter of 64 mm +/- 2 mm*). In contrast gc conidia germinated after 7 to 8 d under the same conditions, and of 69 isolates prepared from two strains each derived from single ascospores, all produced typical gc colonies. Twenty second generation conidial isolates from a wf colony (i.e. isolates arising from a wf conidial isolate) showed little variation in appearance and were similar to the parental colony except that the wf and ws zones were smaller, extending to diameters of 23 mm (+/- 2 mm*) and 53 mm (+/- 6 mm*) respectively.

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* +/- x 95% confidence intervals.

Examination of the mycelial type of colonies arising from single hyphal tips excised from wf or ws mycelia from wf and wf/ws colonies respectively, showed that both sorts of hyphae were capable of generating wf and wf/ws colonies (Table 3.10). A large proportion of all hyphal tip colonies were wf/ws irrespective of their source. Even hyphal tips from wf mycelia (at 14 d) produced mostly (8/10) wf/ws colonies and tips isolated from the same colony after 21 d all resulted in this colony type. Colonies that were completely wf occurred with much lower frequency. For instance 2/10 tip colonies from wf hyphae (wf colony) at 14 d were completely wf, 3/10 from ws hyphae (wf/ws colony) at 14 d and 1/10 from ws hyphae (wf/ws colony) at 21 d. Also there seemed to be a tendency away from wf, to wf/ws or ws colonies the more mature (i.e. 21 d) the original colonies from which hyphae were excised. Totally ws colonies only arose twice, both from ws hyphae of a 21 d wf/ws colony.

Just as subcultures and/or conidial isolates from ws, wf and gc mycelia consistently produced colonies with the mycelial morphology of the parental type, colonies arising from ws, wf and gc inocula were always ws, wf and gc respectively even when they were incubated under conditions of darkness and 12 h light/12 h dark (Table 3.11). Colonies arising from the same inocula but incubated under different treatments did not appear to differ markedly from one another, except that incubation in continuous light appeared to inhibit growth of ws and wf colonies.

iii. Development of pigmentation in Hypoxylon fragiforme colonies

The development of pigmentation was compared in H. fragiforme colonies that were incubated under conditions of darkness (D), 12 h light/12 h dark (L/D) and light (L) (Figure 3.14). These results were not subjected to statistical analysis as they were only regarded as preliminary. The pigmented area was expressed as a percentage of colony size. Pigment developed in colonies under all treatments, but exposure to light enhanced pigment production. Hence colonies kept in D, L/D and L after only 5 d incubation had 6%–21%, 32%–58% and 42%–68% pigmentation and finally after 35 d incubation had 37%–74%, 88%–100% and 75%–100% respectively. Although initially (5 d) colonies in the L had higher pigmentation values than those in L/D, there did not appear to be a correlation between length of exposure to light and percentage pigmentation. This was because later (14 d, 21 d and 35 d) three (as1, w1 and w2) of the five strains had higher values in the L/D than in the L regime.

Between 5 d and 7 d the percentage pigmentation appeared to drop slightly in strains as2, as3 and w2 in L/D, and as3 and w2 in L, in contrast to the gradual rise in pigmentation observed for other strains. A similar drop in percentage pigmentation seemed to occur in as2 and w2 in D between 7 d and 14 d. This drop may have been due to an increase in linear extension rate of colonies without a corresponding increase in pigmented area. The situation however was resolved (i.e. percentage pigmentation increased with time) by 14 d in L and L/D and by 21 d in D treatments.

In L and L/D treatments the greatest increase in percentage pigmentation occurred between 7 d and 14 d and, after the latter, tended to level off. In contrast, under D, pigmentation increased the most between 14 d and 21 d and in all strains, except as2, continued to rise gradually up to 35 d.

Generally there was little variation in percentage pigmentation between strains, even between those from wood and single ascospores, although as1 and w2 were consistently less pigmented in L and D treatments than other strains.

Examination of pigment development in one strain (as3) following exposure to limited periods of light resulted in observations that agreed with those recorded above (Figure 3.15). Firstly the percentage pigmentation was lower in colonies that were kept in darkness than those that were illuminated. Secondly with regard to illuminated colonies the length of the light period did not appear to have a direct effect on the amount of pigmentation. Hence colonies exposed to 6 h and 9 h light had lower percentage pigmentation values than those exposed to 3 h. However, as above, these results were not subjected to statistical analysis as they were regarded as a preliminary investigation. A drop in percentage pigmentation from 2 d to 9 d following illumination may have occurred because linear extension rate exceeded pigment production. The final values for percentage pigmentation at 35 d following illumination (i.e. 40 d total incubation) ranged from 55% to 69% which were a little higher than the maximum value at 35 d incu-

bation obtained for the same isolate in Figure 3.14, treatment D.

iv. Phenol oxidase and peroxidase activity

The development of colour in the agar and mycelium around substrate wells containing phenols was regarded as an indication that the mycelium possessed phenol oxidase, that is laccase (for the phenols salicylic acid, quinol, catechol, guaiacol and ferulic acid) or tyrosinase (for the phenol dihydroxyphenylalanine) activity. Where colours developed they did so between 3 h and 6 h after the wells were filled and the colours observed at 6 h were the same as those recorded at 18 h. Colours varied from pinks (e.g. rosy buff and rose - Figure 3.16), oranges (e.g. cinnamon and brick) and brown (e.g. isabelline and chestnut) and where they did not develop "no reaction" (NR) was recorded (Table 3.12).

With regard to unpaired mycelia none of the 12 species tested developed colour with salicylic acid and only one strain (as2) of H. multiforme and one strain of R. desmazieresii (w1) produced a reaction with ferulic acid and dihydroxyphenylalanine respectively. By contrast colour was recorded around the quinol well in seven species, D. concentrica, H. mammatum, H. multiforme, H. nummularium, "H. purpureum", H. rubiginosum and R. mammiformis, although sometimes only in one of two or three strains. In D. concentrica, H. nummularium, "H. purpureum" and H. rubiginosum, quinol was the only substrate that elicited a response. Colour also developed around the wells of catechol and guaiacol in H. fragiforme, H. mammatum, R. desmazieresii and R. mammiformis for

the former and H. mammatum, H. multiforme, R. desmazieresii and Xylaria polymorpha for the latter. Although results were consistent for all strains of some species (e.g. both strains of D. concentrica only reacted to quinol) in other species different strains gave different results (e.g. one strain of H. fragiforme reacted with catechol but the remaining three did not react with any of the six substrates). None of the phenols brought about a response in any strains of H. serpens or Xylaria longipes.

All species tested possessed peroxidase activity and the amount, although recorded on an arbitrary scale, seemed consistent for strains of each species, but varied between some species. Mycelia of H. fragiforme, H. mammatum, H. multiforme, "H. purpureum", R. mammiformis and gc and wf mycelial types of H. serpens only had limited peroxidase activity (+). Mycelia of ws H. serpens, H. rubiginosum and X. polymorpha had slightly higher activity (++). Rosellinia desmazieresii, X. longipes and H. nummularium mycelia had the highest activity (+++).

3.4 Discussion

The cultural characters and colony ontogeny of mycelia of the Xylariaceae studied here have shown that each species has distinctive features, such as colony extension rate and mycelial colour and texture, that distinguish it from others. Within some species, such as Hypoxylon fuscum and Hypoxylon fragiforme, some variation in these characters was demonstrated even between single ascospore isolates from the same progeny set. By contrast isolates

of other species, such as Hypoxylon multiforme were remarkably uniform within progeny sets and also often between progeny sets. This may indicate that the sexual reproductive cycles in these two groups of species are different. These will be considered further in Chapters 4 and 5.

In their discussions of developmental versatility in fungal mycelia, Rayner and Coates (1987) and Rayner, Boddy and Dowson (1987) proposed that at least five switch mechanisms occur which regulate the adoption of alternate morphogenetic modes. These switches are often superimposed on one another and may be expressed in a continuum so that abrupt alterations in mycelial morphogenesis do not occur: rather the mycelium gradually changes its character as growth proceeds. However, in some cases, either in response to exogenous or endogenous cues, abrupt transitions between radically differing, often reciprocal, developmental patterns occur. Such transitions result in the dimorphisms or polymorphisms between alternate modes which provide the most direct evidence for the operation of switching systems in fungi grown in artificial culture.

The switches postulated by Rayner and Coates (1987) and Rayner, Boddy and Dowson (1987) were determinate-indeterminate transitions, alterations in internode length and branch angle of hyphae, aerial versus appressed or submerged growth, compacted versus diffuse morphogenesis and "juvenility" versus "senescence". Determinate (unicellular)-indeterminate (filamentous) transitions occur at

spore germination, in reverse at sporogenesis and in a number of fungi between yeast and mycelial forms. The ecological significance of the latter dimorphism lies in the advantages of the yeast form in stress-tolerance and dispersion in mobile media, prior to the rapid exploitation by the mycelium, which can colonize fixed spatial domain once conditions are improved. For example several plant pathogens initiate colonization as yeasts in the sapstream and revert to mycelial development as the host becomes weakened by the effect of pathogenesis or stress. Alterations in internode length can change the balance between extension growth and branching, and variation of branch angle can provide different degrees of alignment of hyphae. These changes are demonstrated in the maturation of a colony from a germinating spore, where there is a progression from initially divergent growth, characterized by small internodes (closely spaced branching) and wide angles (a "slow-dense" mode), to increasingly outwardly directed growth with large internodes and smaller angles (a "fast-effuse" mode). These adjustments have clear implications for the colonization process, the slow-dense mode aiding initial establishment, resource exploitation and consolidation of territorial domain, whilst the fast-effuse facilitates exploration and coverage of domain. In the aerial/appressed growth dimorphism, the aerial mycelium acts as a drain on resources from hyphae in contact with the substratum, as well as a means of liberating hyphae from the constraints imposed by the physical boundaries of the substratum, staling products or poor aeration. The divergent growth of hyphae is superseded by convergent growth in diffuse versus compacted morphogenesis, res-

ulting in hyphal fusion and aggregation. The exact form of the plectenchymatous structures that are generated such as sclerotia, fruit body primordia, pseudosclerotial plates and mycelial cords or rhizomorphs, depend on whether compaction is generalized or localized and the mycelial form on which it operates. Finally, the alteration between "juvenility", usually marked by well-developed aerial growth, and "senescence", indicated by often pigmented mycelium in which radial and/or aerial growth is suppressed, is usually unidirectional. The functional role of this transition may concern the redirection of growth in preparation for adoption of a new morphogenetic mode.

Single ascospore isolates of many species in the present study such as Hypoxylon nummularium and Daldinia concentrica produced a more or less uniform mycelial mat, that is zones or sectors of different mycelial types were not produced. This is probably because these species express their distinctive morphological options in a continuum, and either do not possess the ability to switch between distinctive reciprocal modes of development, or had not been exposed to suitable endogenous or exogenous stimuli to allow them to do so. However, the mycelium of single ascospore isolates of some species exhibited distinctive patterns of development even under homogeneous culture conditions, producing sectors (e.g. H. multiforme) or annulations (e.g. concentrically zoned (CZ) colonies of "Hypoxylon purpureum") of mycelium that were morphologically distinct from the rest of the colony. Such behaviour provided evidence for spatiotemporally discontinuous

expression of distinctive mycelial modes. These will now be discussed whilst emphasising at the outset that these ideas are in their infancy.

In Hypoxylon serpens there is evidence for modulation between three distinctive options of morphogenesis which are aerial/non-aerial, slow-dense/fast-effuse and determinate/indeterminate (Figure 3.17). On the basis of the present evidence the primary option appears to be between aerial (superficial) and non-aerial (appressed/submerged) growth giving rise to white silky (ws) and grey conidial (gc) mycelial types respectively. Ascospore isolates from single perithecia seemed to be committed to this inbuilt dimorphism as they were either ws or gc. As yet there is no evidence from cultural studies that there can be a complete switch from one form to the other. However, this is not to say that such a switch may not occur in nature (see below).

Although the aerial/non-aerial (ws/gc) dimorphism appears to be irreversible, there was evidence that ws could spontaneously change into a possibly intermediate white felty (wf) form following sub-culture after prolonged incubation. This change from ws to wf mycelia was clearly brought about by a fast-effuse/slow-dense dimorphism, that is by an alteration in internode length and branch angle; relatively long internodes and small branching angles (26°-31°) characterizing ws (fast-effuse) and shorter internodes and wider angles (50°-56°) in wf (slow-dense). The spontaneous reversion of wf to ws mycelia is a phenomenon referred to as

point-growth (Rayner et al., 1985) and this, and the origin of wf from ageing ws, is reminiscent of a similar fast-effuse to slow-dense mode switch recorded in the basidiomycetes Hypholoma fasciculare (Rayner and Dowson, pers. comm) and Serpula lacrimans (Coggins et al., 1980). Further, evidence for this switch was provided in the present study when "velvety slow" reverted to "cottony fast" mycelia of Rosellinia mammiformis, and "typical" buff or honey mycelial fans emerged from dull green or isabelline pigmented mycelium of H. fuscum.

Associated with the fast-effuse/slow-dense dimorphism of ws and wf H. serpens mycelia was an indeterminate (filamentous)/ determinate (unicellular i.e. conidial) switch, whereby ws mycelium never became conidiogenous, but wf did, spreading from the inoculum outwards. Corresponding to this was the observation that ws mycelium was the only form able to produce pseudosclerotial plates (PSPs). This separation of a fast-effuse non-conidial PSP-producing mycelial type and a slow-dense condial form again appears to be similar to equivalent modes (mentioned above) in Hypholoma fasciculare (Rayner, 1975; Rayner et al., 1985). It is possible that the association of PSP production with fast-effuse mycelium and conidiogenesis with slow-dense mycelium may be related to the alignment of hyphae in these two forms. Hyphae in fast-effuse mycelium perhaps lie side by side so that they may be easily compacted together into tightly packed sheets, whilst the dense network of much-branched hyphae in slow-dense mycelium are less able to be compacted and instead become conidiogenous.

The wf mycelial type is perhaps an intermediate form between ws and gc as it appears to share common features with both types. It arises from ws and like it is aerial and initially white, but it becomes conidiogenous and grey and has a slow-dense colony form like gc. The growth features of gc leader hyphae often at least partly coincided with those of wf whilst differing from ws. The irregular patterns, for example in the length of successive branches, of gc hyphae may have been due to the limited number of second and third branches measured, as these were often obscured by conidiophores and conidia. Although switches linking the gc state to either wf or ws were not directly observed in culture, ws and gc mycelia do develop adjacent to one another on wood (see Figure 3.2).

This behaviour is reminiscent of the striking aerial-appressed dimorphism in Hymenochaete corrugata in which the two colony forms (a white aerial - "fluffy" - and a yellow-brown pigmented appressed - "flat" - form) with similar extension rates, develop together on the natural substratum. The appressed type is associated with more decayed regions (Sharland, Burton and Rayner, 1986), whilst the aerial form only is capable of formation of PSPs. Superimposed on this aerial-non-aerial dimorphism is a slow-dense/fast-effuse dimorphism, the distinction between aerial and non-aerial being accentuated in the slow-dense mode.

Ascospore isolates of H. serpens that were gc always originated from stromata on wood in an advanced state of decay compared to

wood bearing stromata that yielded ws isolates. This indicates that in this species the gc state like the appressed form of H. corrugata is associated with more decayed regions. This information, coupled with the advantages that the ability of a mycelium to alternate between a fast-effuse and a slow-dense mode would have during colonization (see above) (Rayner and Coates, 1987; Rayner, Boddy and Dowson, 1987), combine to suggest a link between the mycelial types of H. serpens observed here and their possible role in nature. The ws mycelial type, with its fast-effuse exploratory mode of growth may establish the fungus in a resource, enabling it to spread rapidly outwards in the search and capture of new domain. It is possible that perhaps this is the mode in which lignocellulosic materials are actively degraded as ws mycelium showed more peroxidase activity (++) than either wf or gc (+) (these differences however may not be very meaningful as the method of assessment was arbitrary). However, none of the mycelial types, ws, wf or gc produced positive results when tested for laccase or tyrosinase activity. Why this should be is not entirely clear, but is discussed further below.

It may be significant that the ws mycelium has the ability to produce PSPs - structures facilitating defence of territory obtained by primary resource capture (Cooke and Rayner, 1984; Rayner et al., 1985). Later the ws mycelium may switch, via an initial wf mycelial mode to the gc state. In this context it is of interest that conidia of Hypoxylon species are usually produced on the surface of the ectostroma in the early stages of stromatal

development prior to perithecia and ascospore formation (Miller, 1961).

Although the hypothesis proposed above may provide the basis for a plausible explanation of the adaptive significance of the mycelial types, it should be pointed out that H. serpens is regarded as a very variable species. Indeed, as a result of detailed taxonomic studies it is now regarded as a complex comprising several separate species (Pouzar, 1985a, b; Petrini and Rogers, 1986). The ws and gc isolates collected here were obtained from stromatal material that appeared to be superficially identical, and dimensions of ascospores from separate samples did not differ markedly from one another. Other characters such as the ascus reaction with iodine, or the morphology of the ascospore germ slit, that may be critical taxonomically, however were not examined as they did not concern the primary aim of the present investigation. It may be that detailed taxonomic analysis of these samples would reveal them to be distinct species within the H. serpens complex. This being so, the findings reported here in this preliminary study are nevertheless important, as they indicate that there is phenotypic plasticity, at least for example in the ws mycelial type. In addition the possibility that the wf mode may be an intermediate mycelial type between ws and gc indicates that further investigations into mycelial development in H. serpens are required. Cultures of separate species within the complex have been examined and ws, wf and gc colony types were represented.

Like H. serpens, Hypoxylon rubiginosum is regarded as highly variable particularly with respect to stromatal characters (Miller, 1961). Indeed following a thorough investigation of a great deal of material and taking earlier studies (such as those of Greenhalgh and Whalley (1970) and Whalley and Greenhalgh (1971)) into account, Petrini and Müller (1986) recognized three distinct varieties of H. rubiginosum. In the present study since "H. purpureum" stromata bore a superficial resemblance to those of H. rubiginosum and the ascospore dimensions of both were in the same range, "H. purpureum" might reasonably be assumed to be a variant of H. rubiginosum. However, the cultural characters of "H. purpureum" mycelium in both "typical" (T) and concentrically zoned (CZ) colonies were clearly distinct from those of the 88 isolates from 11 samples of H. rubiginosum which appeared to be remarkably uniform, even between samples. Further, the cultural characters of "H. purpureum" do not appear to be represented by those of any of the three H. rubiginosum varieties proposed by Petrini and Müller (1986). As for H. serpens a detailed taxonomic study of the samples of "H. purpureum" is needed.

Another parallel with H. serpens is that several switches appear to be involved in the occasional conversion of T to CZ "H. purpureum" colonies. Presumably the primary option is a slow-dense/fast-effuse (T/CZ) dimorphism although details of such a change were not examined. That several morphologically distinct mycelial modes were expressed in CZ colonies and that the sequence in which these were produced varied, suggests that once development follows

the fast-effuse option a further range of possibly associated dimorphisms becomes available. These may include indeterminate (filamentous)/determinate (conidial) development (e.g. white downy to cottony mycelium - WDC/buff conidial-BC), aerial/appressed growth (e.g. dull green - DG - or mounds of BC/WDC and BC) and "abnormal" lateral branching/"normal" branching on aerial hyphae (e.g. DG/WA).

It is possible that, as suggested above, for fast-effuse/slow-dense mycelia of H. serpens, in nature WDC and WA mycelia of CZ colonies may be involved in the initial exploration and capture of domain, whilst the abnormally branched DG mycelium of T colonies may be a form in which established domain is consolidated. Indeed, the dense network or abnormally branched aerial hyphae and associated strong, bitter-sweet smell are perhaps characters that equip the DG mycelium for combat against other fungi that may invade its domain.

An intriguing feature of the alteration between T and CZ colonies concerned the production of pigment either in the medium, or on the surface of the mycelium. The slowly extending T forms produced extensive pigment in the medium, whilst the more rapidly extending CZ forms exuded brown liquid droplets onto their mycelial surface. It would be interesting to characterize the pigment and liquid to establish if they are the same, to test for its possible effect on colony radial extension rate and perhaps to identify any link between this and the bitter-sweet smell.

The unrestricted (U) and restricted (R) mycelial types of H. fragiforme and H. multiforme appear primarily to involve a dimorphism between juvenile and senescent growth; the well-developed aerial growth (U) and the areas where radial and aerial growth (R) appear to be suppressed, correspond to the juvenile and senescent modes respectively. That internode lengths of R hyphae were consistently shorter than those of U hyphae, whilst branching angles were not markedly different, supports this view of a juvenile/senescent dimorphism, as opposed to a slow-dense/fast-effuse switch where both growth parameters may be expected to be altered. The pigmentation associated with the R mycelial type, particularly apparent in H. fragiforme, is also consistent with a senescent phenomenon (Rayner and Coates, 1987). However, the shape of the senescent regions differed between the two species. In H. fragiforme growth was arrested either along the entire colony margin, resulting in senescent rings, or radiating from a single point, producing sectors. By contrast, senescence in H. multiforme was confined to sectors. These patterns are similar to those of "ring" and "sector" differentiation that develop randomly from small brown areas in the submarginal zone of juvenile mycelia in Nectria haematococca. A specific cytoplasmic determinant is associated with each state (Daboussi-Bareyre, 1980). Extra-chromosomal elements are implicated as effecting transitions to senescent states in several Deuteromycotina and Ascomycotina (Rayner and Coates, 1987). It is possible that this may be the case in H. multiforme and H. fragiforme, but evidence for transmission

of senescence to juvenile forms, needed to substantiate this possibility, was not sought during the present study.

That the hyphal growth parameters associated with R and U modes in both H. fragiforme and H. multiforme were distinctive within any particular strain, but overlapped between different strains may be accounted for by the expected variability between strains.

The lack of stability between mycelial modes in these xylariaceous species discussed above (demonstrated between all developmental states except between ws and gc H. serpens) is not unique, but is a feature of mode-switches in several fungi exhibiting dimorphisms or polymorphisms. For example the relatively slow-extending powdery mutant ("up-mut") and the more rapidly extending fibrous-striate "wild-type" colony forms of the slow-dense/fast-effuse dimorphism of strains of the EAN aggressive race of Ophiostoma ulmi, can change from one form to another following subculture (Brasier, 1986). Indeed, the "up-mut" form can revert to the wild type by point-growth, just as H. serpens wf reverts to ws. Another example is the interconversion between some of the five morphological variants of Fusarium oxysporum which appear to involve a primary aerial/appressed growth dimorphism supplemented by secondary options much as in H. serpens. The variants are sporodochial (sp), cottony (co) and ropy (ro) all with abundant aerial growth and slimy pionnotal (slp) and shorn (sh). These forms are often fairly stable, but they can interconvert for example,

between sp to co or slp, especially when aged inocula are used. A genetic regulatory mechanism is implicated (Burnett, 1984).

Studies of the effect of an external factor, light, on the developmental switches between T and CZ colonies in "H. purpureum" and ws, wf and gc mycelia of H. serpens, revealed that only in "H. purpureum" was interplay with endogenous controls evident. Colonies arising from WA inocula from T colonies that were exposed to light resulted in CZ-like colonies (light-induced CZ - LICZ - colonies) but these differed from CZ colonies produced in darkness in several respects. Temperature seemed to have no effect on the switches, although radial extension rate and aerial growth of both colony types were restricted or inhibited at 5°C and 30°C and above.

Returning to the effect of light, growth of H. serpens ws and wf mycelia was inhibited by continuous exposure, whilst gc mycelia appeared to be unaffected. It may be that pigmentation of the latter protects the hyphae and conidiophores against light-induced damage, which in the non-pigmented ws and wf mycelial types prevents extension and aerial growth.

Pigmentation may also play a similarly protective role in H. fragiforme. In support of this view, exposure to light appears to accelerate pigment production. A dose/response relationship was not evident. It therefore seems likely that light triggers an "on/off" switch for pigment production that would otherwise be effected by purely endogenous controls. Presumably the observed variation in

the capacity of individual strains to produce pigment (such that as1 and w2 consistently yielded less than other strains, irrespective of treatment) is a result of genetic differences between strains. These may also account for differences in laccase or tyrosinase activities between individual strains of particular species discussed below.

The twelve species examined for phenol oxidase activity fell into three groups: those that appeared to produce neither laccase nor tyrosinase (H. serpens and Xylaria longipes), those producing both (Rosellinia desmazieresii) and those producing only laccase (D. concentrica, H. fragiforme, Hypoxylon mammatum, H. multiforme, H. nummularium, "H. purpureum", H. rubiginosum, R. mammiformis and Xylaria polymorpha). That the majority of species fell into the final group is not unexpected, as all members of the Xylariaceae have the capacity to degrade lignin and cellulose, that is to cause a white rot (Rogers, 1979a). In an examination of oxidation reactions with phenolic compounds by wood-decay fungi, Käärik (1965) included D. concentrica, Ustulina deusta and X. polymorpha in the group which she referred to as "real white rot fungi".

That H. serpens and X. longipes seemed to produce neither laccase nor tyrosinase, a feature perhaps of brown rot fungi, is surprising. It may be that the age of the mycelium tested affected the results. Käärik (1965) found that for most of the fungi she tested there were no changes in reaction observed between young vigorously growing mycelia (10-20 d old) and older mycelia (30-80 d

or 100-120 d) but in some fungi results with older mycelia were essentially changed. For this reason in the present study mycelia were usually tested as soon as possible after isolation from wood or ascospores, but some strains, including those of H. serpens and X. longipes, had been repeatedly subcultured (and those of X. longipes had been stored for at least 2 years under liquid paraffin).

Differences in laccase or tyrosinase activities between individual strains of the same species, so that for example one strain of H. fragiforme (as1) produced colour with catechol, whilst the others (as2, w1 and w2) did not react at all, is not unusual. Some species (e.g. Hypholoma fasciculare) consistently produce the same activity response irrespective of strain, whilst others display considerable variation in activity between strains (Käärik, 1965). For example strains of Peniophora gigantea and Stereum sanguinolentum showed very variable results. In S. sanguinolentum monospore isolates from two different sporophores showed that although most isolates from each sporophore produced similar reactions, those from different sporophores differed markedly and it was concluded that these differences were probably fixed genetically.

Differences between species in the amount of peroxidase activity they displayed cannot be considered as very meaningful since the assessment method was arbitrary and levels of such activity may be altered by the physiological state of the mycelium.

However, that all twelve species possessed peroxidase activity is in agreement with previous findings such as those of Law (1950) in which all white rot fungi tested gave a strong peroxidase reaction.

There was no real evidence for compartmentalization of enzyme activity between distinctive mycelial types of H. fragiforme or H. serpens, but this should be re-examined. By contrast, in "H. purpureum" the T colony that was tested had laccase activity whilst the CZ had none. This possible partitioning between the two developmental states requires substantiation through examination of a range of strains expressing both modes, as the difference recorded here may be due to genetic differences in the strains tested. That enzyme activity may be so compartmentalized is quite likely. In some hymenomycetes laccase activity has been shown to be restricted to the mycelium, whilst tyrosinase activity is confined to the sporophores (Lindeberg and Holm, 1952; Lindeberg, 1950). Moreover, the similar compartmentalization between different developmental states of the mycelium in Phellinus tremulae (Hiorth, 1965) and Hymenochaete corrugata (Sharland and Rayner, unpublished) has already been mentioned.

It is evident from the striking examples of developmental variability in culture of some of the xylariaceous species studied here, that the endogenous factors controlling the observed switching of mycelia between distinctive developmental states are finely balanced, and responsive to both endogenous and/or exogenous

stimuli. With regard to the latter, sometimes it appears that extremely subtle changes in the environment elicit a response. Although proposals as to the possible functions of these dimorphisms/polymorphisms have been put forward, their precise mechanism and function require further investigation. This, together with examination of the other species that did not express distinctive mycelial types, to establish if they too have the potential to exist in a variety of modes, would be helpful as it may aid understanding and interpretation of the ecological behaviour of these species.

Table 3.1. Cultural morphology and growth rates of xylariaceous fungi grown on 2% malt extract agar at 20°C in darkness.

Colours and textures are described using the terminologies of Rayner (1970) and Stalpers (1978) respectively.

Species	Mean daily radial extension rate (mm)	After 7 d incubation				After > 21 d incubation			
		Texture	Margin	Colour From above	Colour From below	Texture	Margin	Colour From above	Colour From below
Daldinia concentrica	5.9–8.0 (ascospore isolates)	cotton to cottony-woolly	appressed and even	white	white	Woolly, granular with numerous minute flecks. Some cultures floccose due to development of white mycelial tufts. — all ascospores & some wood & stromatal isolates	–	Pale olivaceous grey or olivaceous grey with olivaceous grey to iron grey flecks.	Honey to isabelline to dark mouse grey/fuscous black spreading outwards from inoculum.
	5.2–7.2 (wood isolates)					Zonate with felty conidial mat. — remainder of wood & stomatal isolates	–	As for woolly granular but conidial mat vinaceous buff to hazel.	As for woolly granular colonies but less darkening.
Hypoxylon fragiforme	4.5	woolly	even, except in some isolates (see text)	white with ring around inoculum of isabelline/ pale mouse grey or ochraceous/ isabelline or pistachio green	white with ring around inoculum of isabelline or ochraceous/ isabelline or dark bluish green	Woolly to sub felty. After > 56 d some isolates produced conidiomata, others developed floccose conidia-bearing areas.	–	Occasionally white, more usually buff, or buff with green olivaceous flecks. Conidial areas sulphur yellow to pure yellow.	Buff to honey sometimes mottled with cinnamon flecks or fans. Sienna/ umber and/or dark bluish green or dark herbage green around inoculum.
Hypoxylon fuscum	2.1	pellicular of velvety	even, except in some isolates (see text)	buff or honey	buff or honey	Pellicular or velvety. After > 80 d often powdery. Conidial areas produced particularly around inoculum.	–	Colours varying from rosy, isabelline, olivaceous, grey olivaceous or dull green to citrine green spreading outwards from inoculum. Elsewhere buff or honey. Conidial areas vinaceous buff to fawn.	

Table 3.1. (continued).

Species	Mean daily radial extension rate (mm)	After 7 d incubation				After> 21 d incubation			
		Texture	Margin	Colour From above	From below	Texture	Margin	Colour From above	From below
Hypoxylon mammatum	3.2	cotton-woolly	irregularly lobed	white		Cotton-woolly with tufts or aggregations of aerial mycelium particularly at the margin. Some colonies floccose with aggregations throughout. A few isolates produced small irregular conidial patches around inoculum.	-	White, where conidia produced pale mouse grey to mouse grey.	
ascospore isolates									
wood isolate	3.2	sparse or downy	irregularly lobed	white		Sparse and downy but more mycelium in vicinity of inoculum.	-	Luteus patches around inoculum. Elsewhere white.	
Hypoxylon multiforme	6	downy to woolly	even, except in some isolates (see text)	white		Woolly to plumose.	-	Buff to honey and around inoculum greyish sepia and then olivaceous buff with greenish glaucous patches spreading (after 35 d) over whole colony.	Olivaceous to dark mouse grey in centre fading to isabelline at edges.

Table 3.1. (continued).

Species	Mean daily radial extension rate (mm)	After 7 d incubation				After > 21 d incubation			
		Texture	Margin	Colour From above	Colour From below	Texture	Margin	Colour From above	Colour From below
Hypoxylon nummularium	6.4-8.5	downy (i.e. colony transparent & composed of fine short erect hyphae) to farinaceous (mealy)	even	white to buff	buff	Farinaceous or woolly. Conidiomata bearing at their apex conidia on nodulose conidiophores with associated brown liquid droplets produced on some isolates at > 80 d.	-	Usually buff, sometimes darkening to honey, or marked by greenish glaucous patches.	Cinnamon or ochraceous in ring around inoculum spreading in time over whole culture. Sometimes darkening to isabelline or sepia.
"*Hypoxylon purpureum*"	1.2	velvety around inoculum, elsewhere silky	even	dull green around inoculum with white band (2 mm) at margin	dull green at inoculum fading to citrine green or through herbage green to white	Velvety over most of colony where mycelium consisted of pigmented hyphae with prolific branches. Margin (< 0.5 cm) silky (no lateral branches). Occasionally some isolates had small silky patches surrounded by velvet areas. All isolates became farinaceous around inoculum where conidia produced on pigmented *Nodulisporium* conidiophores.	even	Buff conidial mat around inoculum surrounded by dull green/iron grey, except for white margin & patches on some isolates.	dull green

Table 3.1. (continued).

Species	Mean daily radial extension rate (mm)	After 7 d incubation				After>21 d incubation			
		Texture	Margin	Colour From above	From below	Texture	Margin	Colour From above	From below
Hypoxylon rubiginosum	1.7	woolly	slightly irregular to even	white		Woolly to felty.	even or irregular where mycelium submerged in agar	Around inoculum grey olivaceous, olivaceous buff, greenish olivaceous or occasionally primrose, sulphur yellow or primrose with pure yellow or saffron yellow edge. From centre colour fades through diffuse greenish glaucous and honey concentric rings to band (3-5 mm) of white mycelium at colony margin.	At centre sepia fading to cinnamon/ buff or isabelline to buff and finally to white at margin.
Hypoxylon serpens "grey conidial"	1.6	Cottony	Even and lobed.	white to smoke grey with a white margin.		Farinaceous where conidia produced on geniculate conidiophores especially around inoculum. Some colonies seemed to grow rhythmically so appressed mycelium alternated with conidial mounds.	even or lobed	Smoke grey darkening to grey olivaceous and then olivaceous grey. Appressed mycelium was smoke grey, conidial mounds were olivaceous grey.	smoke grey
"white silky"	4	silky	Even or occasionally irregularly lobed.	white		Silky. Conidia/ conidiophores absent.	-	White and often mottled with fuscous black pseudosclerotial plates.	

Table 3.1. (continued).

Species	Mean daily radial extension rate (mm)	After 7 d incubation				After > 21 d incubation			
		Texture	Margin	Colour From above	Colour From below	Texture	Margin	Colour From above	Colour From below
Rosellinia desmazieresii	6.4	downy-mycelium sparse and appressed except around inoculum where aerial and woolly	even	white		Cottony to woolly, aerial mycelium spreading from inoculum over whole colony and often aggregated into woolly clumps at edge of Petri dish.	-	White to pale mouse grey, darkening to mouse grey or pale olivaceous grey/ olivaceous grey.	
Rosellinia mammiformis									
"slow velvety"	0.6	velvety	even	white		Velvety	even	white	
"fast woolly"	2.4	woolly	even, regularly or irregularly lobed	white		Woolly around inoculum becoming cottony or silky towards margin, this cottony or silky area becoming conidial.	Irregularly lobed (corresponding) with woolly texture) or even or regularly lobed (corresponding with woolly-cottony/ silky).	white - except pale mouse grey in condial areas	

Table 3.2. Hypoxylon fragiforme. Growth features of restricted (R) and unrestricted (U) mycelial types of strains derived from single ascospores (as) and wood (w) after 5 d incubation at 20°C in darkness.

(+/-95% CI = +/- 95% confidence intervals)

Growth features	Strains											
	as1				as2				as4			
	R		U		R		U		R		U	
	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI
Colony (sector) radius(mm)	15	-	30	-	16	-	26	-	13	-	24	-
Length of leader hypha from tip to first branch (µm)	150	29	615	123	245	55	525	80	114	31	529	86
Leader extension rate ($\mu m h^{-1}$)	18	11	105	36	25	14	92	50	15	7	52	21
Angle between first branch and parent axis (°)	74	10	68	12	71	10	67	10	63	10	61	11
First branch length(µm)	34	17	31	16	56	30	65	40	53	25	42	28
First branch extension rate ($\mu m h^{-1}$)	8	5	36	22	25	11	19	10	12	7	19	10
Relative first branch extension rate (%) $\left(= \frac{\text{first branch extension rate}}{\text{parent hypha extension rate}} \times 100\right)$	44	-	34	-	100	-	21	-	80	-	37	-

Table 3.2. (continued).

Growth features	Strains											
	as6				w1				w2			
	R		U		R		U		R		U	
	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI
Colony (sector) radius(mm)	15	-	22	-	7	-	16	-	8	-	21	-
Length of leader hypha from tip to first branch (μm)	152	57	729	47	295	120	665	140	149	40	492	82
Leader extension rate (μmh^{-1})	17	15	159	49	29	15	120	74	10	5	22	12
Angle between first branch and parent axis (°)	57	13	85	8	62	20	65	10	51	22	63	18
First branch length(μm)	45	28	21	6	32	19	40	11	63	37	33	12
First branch extension rate (μmh^{-1})	20	12	17	7	6	6	3	15	6	5	18	17
Relative first branch extension rate (%) $\left(= \frac{\text{first branch extension rate}}{\text{parent hypha extension rate}} \times 100\right)$	118	-	11	-	21	-	3	-	60	-	82	-

Table 3.3. Hypoxylon fragiforme. Mycelial type of colonies arising from excised hyphal tips from "restricted" (R) and "unrestricted" (U) mycelial types of strains from single ascospores (as) and wood (w).

(The original strains were grown on sterile cellophane overlying 2% MA. All cultures were incubated at 20°C in darkness).

Strain code	Mycelial type of colony (U, R or U/R)	Origin of hyphal tip (U or R)	Mycelial type(s) of hyphal tip colonies after 21 d incubation (total of 10 colonies per mycelial type)			
			U	R	U with R sector(s)	R with U sector(s)
as1	R with U sector	U	10	-	-	-
	U with R sector	R	10	-	-	-
as2	U with R sector	R	8	2	-	-
as3	R	R	-	10	-	-
	R with U sector	U	-	10	-	-
w1	R	R	2	4	2	2
w2	R	R	7	1	2	-
w3	R	R	4	5	1	-

Table 3.4. *Hypoxylon multiforme*. Growth features of restricted (R) and unrestricted (U) colony sectors of strains derived from single ascospores after 5 d incubation at 20°C in darkness.

(+/-95% CI = +/- 95% confidence intervals)

Growth features	Strains											
	1				2				3			
	R		U		R		U		R		U	
	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI
Colony (sector) radius(mm)	20	-	36	-	27	-	32	-	20	-	36	-
Length of leader hypha from tip to first branch (µm)	118	32	273	89	95	28	241	54	140	119	276	78
Leader extension rate ($\mu m h^{-1}$)	16	10	28	9	13	4	23	10	7	10	22	6
Angle between first branch and parent axis (°)	46	23	47	12	52	14	50	13	42	35	49	9
First branch length(µm)	49	17	56	36	29	52	127	68	24	27	25	6
First branch extension rate ($\mu m h^{-1}$)	11	8	13	9	5	4	3	12	3	10	10	4
Relative first branch extension rate (%) $\left(= \frac{\text{first branch extension rate}}{\text{parent hypha extension rate}} \times 100\right)$	69	-	46	-	38	-	13	-	43	-	45	-

Table 3.4. (continued).

Growth features	Strains											
	4				5				6			
	R		U		R		U		R		U	
	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI	mean	+/-95% CI
Colony (sector) radius(mm)	15	-	26	-	19	-	27	-	11	-	24	-
Length of leader hypha from tip to first branch (μm)	195	58	486	90	217	46	545	58	306	100	440	43
Leader extension rate (μmh^{-1})	10	5	66	20	17	13	99	48	43	31	58	17
Angle between first branch and parent axis (°)	46	11	31	12	48	14	43	9	67	13	53	10
First branch length(μm)	54	24	97	52	37	25	50	16	27	7	58	11
First branch extension rate (μmh^{-1})	4	4	18	18	11	11	23	8	14	10	14	7
Relative first branch extension rate (%) $\left(= \frac{\text{first branch extension rate}}{\text{parent hypha extension rate}} \times 100\right)$	40	-	27	-	65	-	23	-	33	-	24	-

Table 3.4. (continued).

Growth features	Strain 7 R mean	+/-95% CI	Strain 7 U mean	+/-95% CI
Colony (sector) radius(mm)	23	-	31	-
Length of leader hypha from tip to first branch (μm)	185	19	216	37
Leader extension rate (μmh^{-1})	8	7	23	8
Angle between first branch and parent axis (°)	30	8	38	14
First branch length(μm)	31	6	46	16
First branch extension rate (μmh^{-1})	12	8	19	12
Relative first branch extension rate (%)	150	-	78	-

$$\left(= \frac{\text{first branch extension rate}}{\text{parent hypha extension rate}} \times 100\right)$$

Table 3.5. *Hypoxylon multiforme*. Mycelial type of colonies arising from excised hyphal tips from "restricted" (R) and "unrestricted" (U) sectors of strains derived from single ascospores (as).

The original strains (selected because they all showed R and U mycelial types) were grown on sterile cellophane overlying 2% MA. All cultures were incubated at 20°C in darkness.

Strain code	Origin of hyphal tip (U or R)	Mycelial type of hyphal tip colony after 7 d and 21 d incubation (total of 10 colonies per sector)			
		U (7 d and 21 d)	U with R sectors (7 d and 21 d)	R at 7 d but U (no R sectors) by 21 d	R at 7 d. U with R sector(s) by 21 d
as1	R	3	4	2	1
as2	U	5	-	5	-
as3	R	3	-	7	-
	U	5	1	4	-
as4	U	9	-	1	-
as5	R	-	-	1	9
	U	2	-	1	7

Table 3.6. "Hypoxylon purpureum". Size (diameter in mm) and type of colonies produced from inocula of different mycelial types after 14 d incubation at different temperatures.

Strain code	Original colony type	Mycelial type of inoculum	Temperature					
			5°C	15°C	20°C	25°C	30°C	37°C
as1	T	WA	NG (white downy growth on inoculum)	T (22)	T (30)	T (16)	BC with cinnamon to umber pigment in agar (5)	NG
c1	CZ	BC	NG	T (26)	T (27)	T (9)	NG	NG
		DG	NG	T (26)	T (28)	T (9)	NG	NG
c2	CZ	BC	NG	T (28)	T (27)	T (11)	BC with cinnamon to umber pigment in agar (4)	NG
		DG	NG	T (26)	T (24)	T (11)	NG	NG
c3	CZ	WDC	NG	CZ (24)	CZ (32)	CZ (13)	NG	NG

Origin of strain/isolate

as ascospore
c conidial (first generation)

Colony types

T "typical"
CZ concentrically zoned
NG no growth

for detailed description see text

Mycelial types

WA white appressed
BC buff conidial
DG dull green
WDC white downy to cottony

Colony diameters (mm in brackets below colony type) are mean values of two replicates.

Table 3.7. "Hypoxylon purpureum". Type of colonies arising from excised hyphal tips from different mycelial types of "typical" (T) and concentrically zoned (CZ) colonies.

The original strains were grown on sterile cellophane overlying 2% MA. All colonies were incubated at 20°C in darkness.

Strain code	Colony type	Origin of hyphal tip (mycelial type)	Colony type after 28 d incubation (10 hyphal tip colonies from each mycelial type)	
			Typical (T)	Concentrically zoned (CZ)
as1	T	WA	7	3
		DG	10	-
as2	T	WA	10	-
c1	CZ	WDC		
		9 d incubation	2	8
		21 d incubation	4	6
		DG	10	-
w1	T	WA	10	-
		DG	10	-
w2	T	WA	10	-

Origin of strain/isolate

as ascospore

c conidial (first generation)

w wood

Mycelial types

WA white appressed

DG dull green (single lateral branches not hyphal tips are isolated)

WDC white downy to cottony

Table 3.8. "Hypoxylon purpureum". Colony type of strains derived from an ascospore (as), a wood (w) and a conidial (c) isolate following 21 d incubation at 20°C in darkness, 12 h light/12 h dark and light.

There were two replicates of each strain per treatment. Where they both produced the same colony type a single description is given. All colony margins were even.

Strain code	Original colony type	Mycelial type of inoculum	Regime		
			Darkness	12 h light/12 h dark	Light
asl	T	WA	T (diameter 36 mm) A_2	CZ (diameter 58 mm) Included central zone (diameter 40 mm) of HF surrounded by WDC. Underneath: replicate 1-sepia, with cinnamon to umber pigment in agar around colony; replicate 2-dull green with citrine pigment in agar. A_1	CZ (diameter 79 mm) Included central zone (diameter 22 mm) of BC surrounded by band (10 mm wide) of HF and WDC to margin. Isabelline to sepia pigment in agar. A_2
w	T	WA	T (diameter 39 mm) A_2	CZ (diameter 44 mm) Included central zone (diameter 40 mm) of HF surrounded by WDC. L around inoculum and underneath colonies olivaceous with cinnamon to umber pigment in agar. A_2	CZ (diameter 66 mm) Included a central zone (diameter 26 mm) of BC surrounded by a narrow (3 mm) band of L on surface of HF and WDC/HF to margin. Replicate 1 remained like this after 35 d; replicate 2 became BC except for WDC margin (7 mm wide). Cinnamon to umber pigment in agar. A_2

Colony types

T "typical"
CZ concentrically zoned

Mycelial types

WA white silky, appressed
WDC white downy to cottony
BC buff conidial
DG dull green - hyphae with prolific lateral branches
HF honey to isabelline, felty textured, becoming buff and conidial after 35 d incubation
L brown liquid droplets

Smells associated with colonies

A_1 faint, sweet smell of roses

A_2 strong bitter-sweet smell, reminiscent of bitter almonds

Table 3.8. (continued).

Strain code	Original colony type	Mycelial type of inoculum	Regime		
			Darkness	12 h light/12 h dark	Light
c3	CZ	WDC	CZ (diameter 42 mm) Included mycelial types BC, DG, WDC, BC, WA from inoculum to margin. A_2	CZ (diameter 31 mm) Included central zone (diameter 23 mm) of HF surrounded by WDC. Underneath olivaceous green to dull green with cinnamon to umber pigment in agar. A_1	CZ (diameter 47 mm) Included central zone zone (diameter 28 mm) of BC surrounded by WDC. Replicate 1 greenish glaucous pigment in agar; replicate 2 cinnamon to umber. A_1

Colony types

T "typical"
CZ concentrically zoned

Mycelial types

WA white silky, appressed
WDC white downy to cottony
BC buff conidial
DG dull green - hyphae with prolific lateral branches
HF honey to isabelline, felty textured, becoming buff and conidial after 35 d incubation
L brown liquid droplets

Smells associated with colonies

A_1 faint, sweet smell of roses

A_2 strong bitter-sweet smell, reminiscent of bitter almonds

Table 3.9. Hypoxylon serpens. Growth features of white silky (ws), white felty (wf) and grey conidial (gc) mycelial types after 10 d incubation at 20°C in darkness.

Growth features	Mycelial type					
	ws		wf		gc	
	Mean	+/- 95% confidence intervals	mean	+/- 95% confidence intervals	mean	+/- 95% confidence intervals
Colony diameter (mm)	58	-	25	-	28	-
Length of leader hypha from tip to first branch (µm)	201	4	148	26	125	31
Internode length between first and second branches (µm)	192	5	160	23	172	50
Internode length between second and third branches (µm)	162	6	147	41	132	43
Angle between first branch and parent axis (°)	26	6	56	10	31	8
Angle between second branch and parent axis (°)	30	9	50	10	62	8
Angle between third branch and parent axis (°)	31	7	No data	-	51	13
First branch length (µm)	37	2	12	4	69	22
Second branch length (µm)	117	6	17	6	43	22
Third branch length (µm)	200	21	No data	-	111	62*

Colonies were grown on cellophane over 2% MA. The figures above are means of a minimum of 30 measurements with exception of details of third branches where only 4(*)-21 measurements were made due to third branches being obscured from view by the dense hyphal network. A leader hypha was the fastest extending hypha in each 1000 µm of colony margin.

Table 3.10. Hypoxylon serpens. Type of colonies arising from excised hyphal tips from "white felty" (wf) and "white silky" (ws) mycelial types of strain D2 derived from a single ascospore.

D2 was grown on sterile cellophane overlying 2% MA. All colonies were incubated at 20°C in darkness.

Mycelial type	Origin of hyphal tip (mycelial type and age of D2 - in d incubated - at time of excision)	Mycelial type(s) of hyphal tip colonies after 28 d incubation (10 hyphal tip colonies from each mycelial type)		
		wf	wf/ws	ws
wf (colony only produced wf mycelium)	wf 14 d	2	8	-
	wf 21 d	-	10	-
wf/ws (colony comprised a central wf mycelial zone surrounded by ws mycelia)	ws 14 d	3	7	-
	ws 21 d	1	7	2

Table 3.11. Hypoxylon serpens. Colony form arising from inocula of different mycelial types following 21 d incubation at 20°C in darkness, 12 h light/12 h dark and continuous light.

There were two replicates of each strain per treatment.

Mycelial type of inoculum	Regime		
	Darkness	12 light/12 h dark	Light
ws (white silky)	ws mycelium covered whole agar surface. A zone (65-70 mm diameter) around the inoculum became olivaceous grey. Conidia absent.	ws mycelium covered whole agar surface.	No growth
gc (grey conidial)	gc, white to smoke grey mycelium to 59 mm diameter. Conidia/ conidiophores sparse. Colony margin lobed.	gc mycelium to 65 mm diameter with an even colony margin.	gc mycelium to 78 mm diameter displaying alternating zones of appressed (pale olivaceous grey, few conidia/conidiophores) and aerial (olivaceous grey, abundant conidia/ conidiophores) growth. Lobed colony margin.
wf (white felty)	Colony (diameter 47 mm) composed of inner wf zone,which became smoke grey and conidial (diameter 21 mm), and an outer ws zone. Colony margin even.	Colony (diameter 48 mm) composed of inner wf zone which became pale olivaceous grey and conidial (diameter 22 mm) and an outer ws zone. Colony margin even.	No growth

Table 3.12. Phenol oxidase and peroxidase activity of some xylariaceous species recorded after 6 h and 30 min respectively.

Species	Strain (mycelial type)	Phenol oxidase						Peroxidase
		LACCASE					TYROSINASE	
		Salicylic acid	Quinol	Catechol	Guaiacol	Ferulic acid	Dihydroxy-phenyl-alanine	
Daldinia concentrica	as1	NR	C (rose)	NR	NR	NR	NR	+
	w1	NR	C (rose)	NR	NR	NR	NR	++
Hypoxylon fragiforme	as1 (U)	NR	NR	C (chestnut)	NR	NR	NR	+
	as2 (U)	NR	NR	NR	NR	NR	NR	+
	w1 (R)	NR	NR	NR	NR	NR	NR	+
	w2 (R)	NR	NR	NR	NR	NR	NR	+
Hypoxylon mammatum	as1	NR	NR	NR	NR	NR	NR	+
	as2	NR	C (isabel-line)	C (isabel-line)	C (rosy buff/ brick)	NR	NR	+

Origin of Strains

a ascospore
w wood
c conidial

Mycelial types

U unrestricted
R restricted
T "typical"
CZ concentrically zoned
wf white felty
ws white silky
gc grey conidial

Reactions

NR no reaction
C Colour development in agar around wells. Colour recorded in brackets.

+ Gas production around well periphery.
++ gas production over whole surface of well.
+++ gas production up to 1 mm above surface of well.

Table 3.12. (continued).

Species	Strain (mycelial type)	Phenol oxidase						Peroxidase
		LACCASE					TYROSINASE	
		Salicylic acid	Quinol	Catechol	Guaiacol	Ferulic acid	Dihydroxy-phenyl-alanine	
Hypoxylon multiforme	as1	NR	C (fuscous black)	NR	C (fuscous black)	NR	NR	+
	as2	NR	C (fuscous black)	NR	C (fuscous black)	C (fuscous black)	NR	+
Hypoxylon nummularium	as1	NR	C (rose)	NR	NR	NR	NR	+++
	as2	NR	C (rose)	NR	NR	NR	NR	+++
"Hypoxylon purpureum"	as1(T)	NR	C (rose - faint)	NR	NR	NR	NR	+
	c1(CZ)	NR	NR	NR	NR	NR	NR	+
Hypoxylon rubiginosum	as1	NR	C (rose)	NR	NR	NR	NR	++
	w1	NR	C (rose)	NR	NR	NR	NR	++

Origin of Strains

a ascospore

w wood

c conidial

Mycelial types

U unrestricted

R restricted

T "typical"

CZ concentrically zoned

wf white felty

ws white silky

gc grey conidial

Reactions

NR no reaction

C Colour development in agar around wells. Colour recorded in brackets.

\+ Gas production around well periphery.
++ gas production over whole surface of well.
+++ gas production up to 1 mm above surface of well.

Table 3.12. (continued).

Species	Strain (mycelial type)	Phenol oxidase						Peroxidase
		LACCASE					TYROSINASE	
		Salicylic acid	Quinol	Catechol	Guaiacol	Ferulic acid	Dihydroxy-phenyl-alanine	
Hypoxylon serpens	as1 (wf)	NR	NR	NR	NR	NR	NR	+
	as2 (ws)	NR	NR	NR	NR	NR	NR	++
	as3 (ws)	NR	NR	NR	NR	NR	NR	++
	as4 (gc)	NR	NR	NR	NR	NR	NR	+
	as5 (gc)	NR	NR	NR	NR	NR	NR	+
Rosellinia desmazieresii	as1	NR	NR	NR	C (rosy buff/ brick)	NR	NR	+++
	as2	NR	NR	C (isabel-line)	C (rosy buff/ brick)	NR	NR	+++
	w1	NR	NR	C (cinnamon - faint)	C (cinnamon /brick)	NR	C (dark brick-faint)	++

Origin of Strains

a ascospore

w wood

c conidial

Mycelial types

U unrestricted

R restricted

T "typical"

CZ concentrically zoned

wf white felty

ws white silky

gc grey conidial

Reactions

NR no reaction

C Colour development in agar around wells. Colour recorded in brackets.

\+ Gas production around well periphery.
++ gas production over whole surface of well.
+++ gas production up to 1 mm above surface of well.

Table 3.12. (continued).

Species	Strain (mycelial type)	Phenol oxidase						Peroxidase
		LACCASE					TYROSINASE	
		Salicylic acid	Quinol	Catechol	Guaiacol	Ferulic acid	Dihydroxy-phenyl-alanine	
Rosellinia mammiformis	as1	NR	NR	C (pale vinaceous-faint)	NR	NR	NR	+
	as2	NR	C (rose)	C (pale vinaceous-faint)	NR	NR	NR	+
Xylaria longipes	as1	NR	NR	NR	NR	NR	NR	+++
	as2	NR	NR	NR	NR	NR	NR	+++
	as3	NR	NR	NR	NR	NR	NR	+++
Xylaria polymorpha	as1	NR	NR	NR	C (brick-faint)	NR	NR	++

Origin of Strains

a ascospore

w wood

c conidial

Mycelial types

U unrestricted

R restricted

T "typical"

CZ concentrically zoned

wf white felty

ws white silky

gc grey conidial

Reactions

NR no reaction

C Colour development in agar around wells. Colour recorded in brackets.

\+ Gas production around well periphery.
++ gas production over whole surface of well.
+++ gas production up to 1 mm above surface of well.

Figure 3.1. Cultural characters of xylariaceous fungi grown on 2% malt extract agar at 20°C in darkness.

(A) Daldinia concentrica. Note the woolly granular texture and flecked appearance of the mycelium.

(B) Hypoxylon fragiforme. The whole mycelium is woolly to sub felty and pigmented after prolonged (56 d) incubation. Note the conidiomata with associated droplets of liquid on their surface.

(C) Hypoxylon fuscum. Note the buff velvety textured mycelium.

(D) Hypoxylon mammatum. Cotton-woolly textured colony with tufts or aggregations of aerial mycelium particularly at the colony margin. Note the pale mouse grey conidial area.

(E) Hypoxylon multiforme. Buff/honey mycelium with greenish glaucous patches and woolly to plumose texture.

(F) Hypoxylon nummularium. Note the buff and woolly textured mycelium.

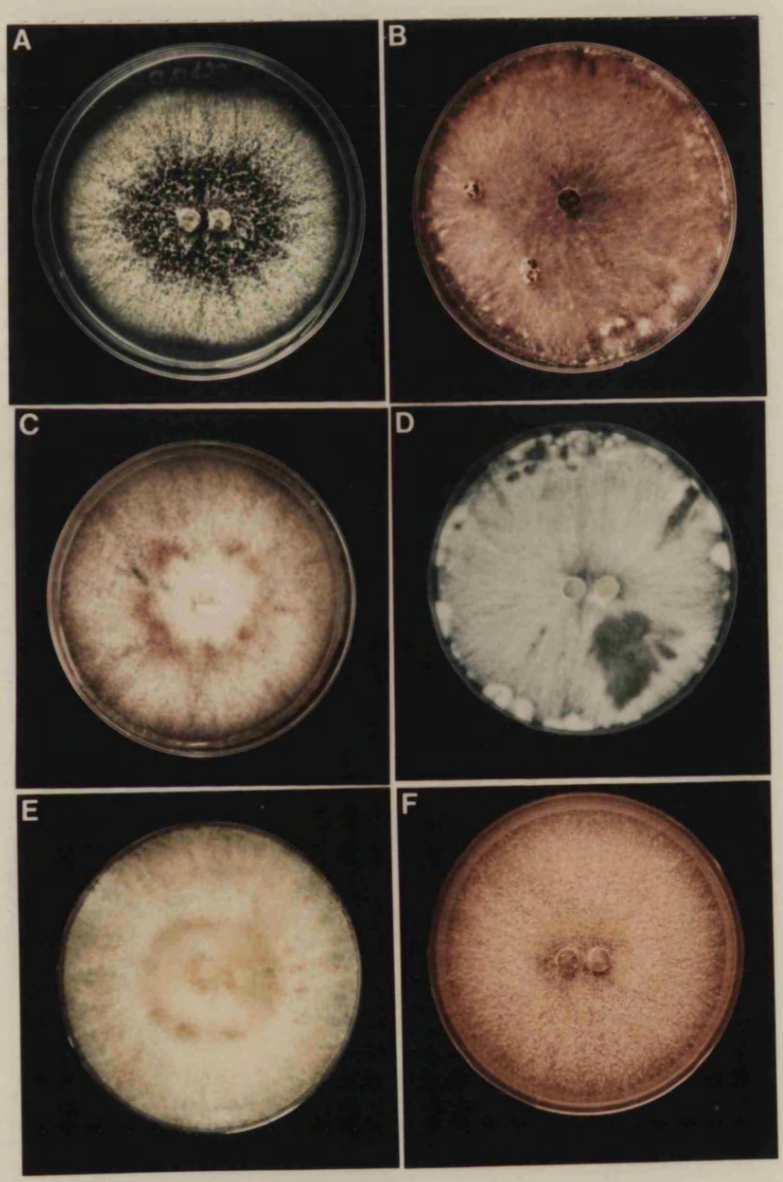

A
B
C
D
E
F

Figure 3.1. (continued).

Cultural characters of xylariaceous fungi grown on 2% malt extract agar at 20°C in darkness.

(G). "Hypoxylon purpureum". "Typical" (T) colony composed of a central buff conidial area surrounded by dull green velvety mycelium of pigmented hyphae with prolific lateral branches and a narrow (< 2 mm) white margin.

(H) Hypoxylon rubiginosum. Woolly to felty mycelium which in the centre is olivaceous buff. The mycelium in the RH colony then alternates between white and pure yellow bands and in the LH colony is pure yellow.

(I) Rosellinia desmazieresii. Sparse cottony mycelium. Note the occasional small spherical aggregations at the colony margin.

(J) Hypoxylon serpens. White silky (**ws**) and grey conidial (**gc**) mycelial types. Note the faster growth rate of **ws** mycelium (**ws** colony incubated for 10 d, **gc** for 14 d).

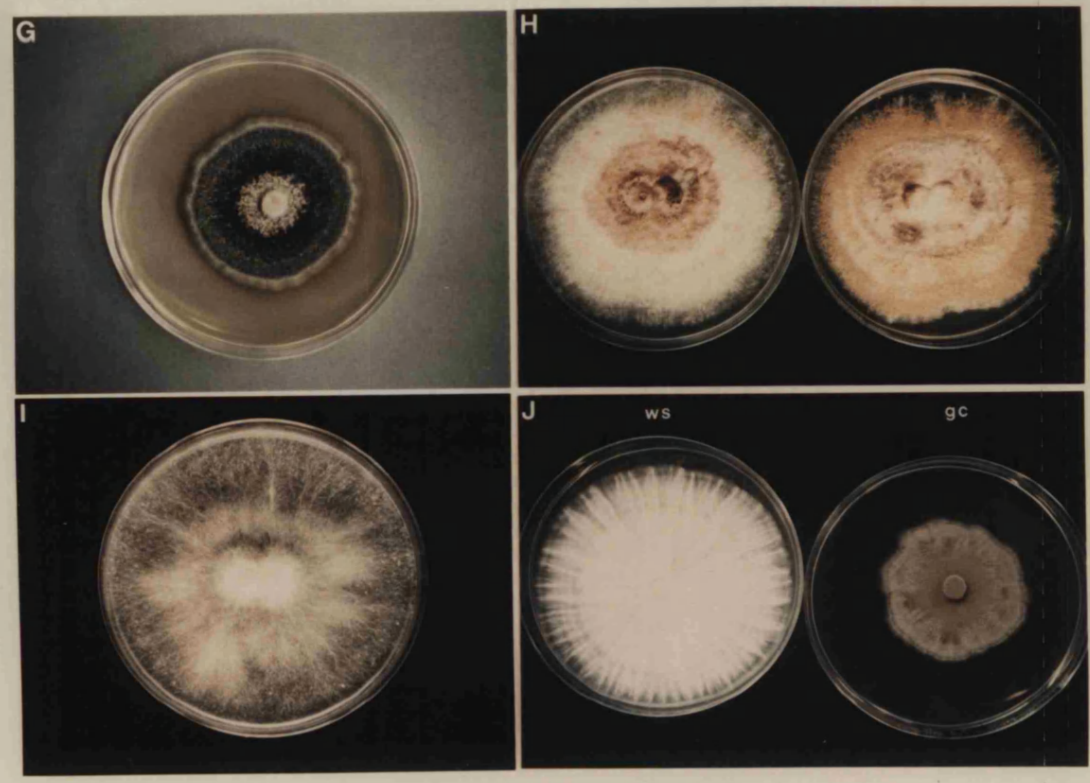

G
H
I
J
ws
gc

Figure 3.1. (continued).

Cultural characters of xylariaceous fungi grown on 2% malt extract agar at 20°C in darkness.

(K) Rosellinia mammiformis. Six isolates showing the range in colony form from "slow velvety" to "fast woolly" mycelium.

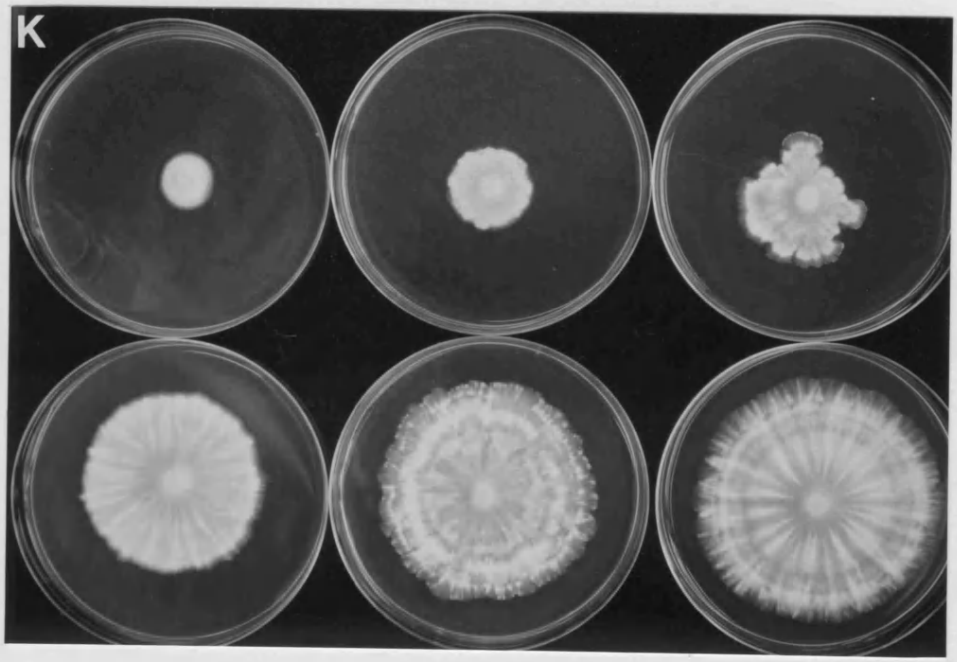

K

Figure 3.2. Grey conidial (gc) and white silky (ws) mycelia have developed adjacent to one another on wood bearing a Hypoxylon serpens stroma, following direct incubation in a polythene bag.

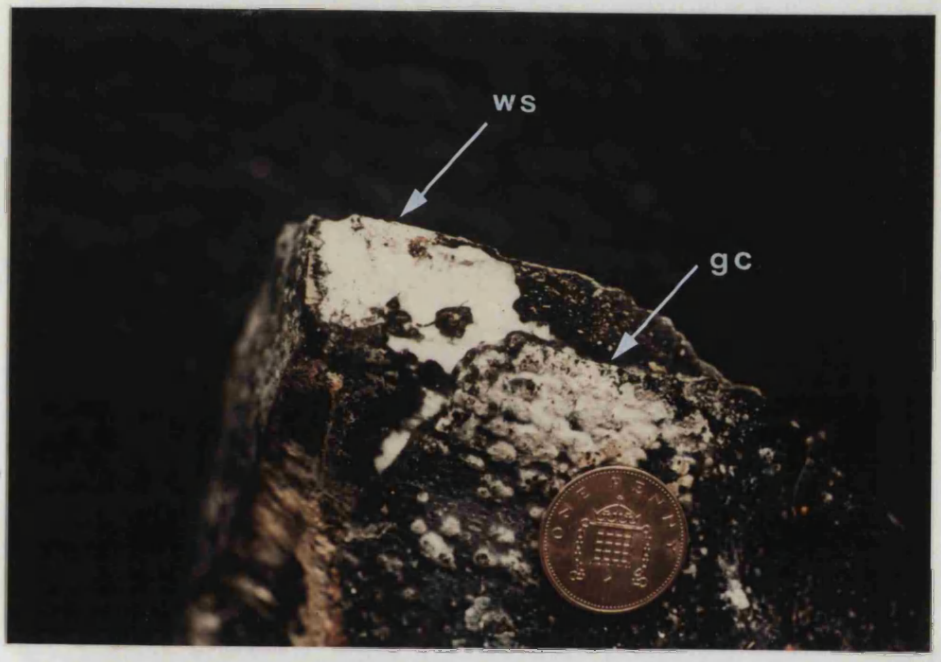

ws
gc

Figure 3.3. Paired isolates of Hypoxylon fuscum in which the RH colony is producing a fast-growing fan of "typical" buff or honey mycelium emerging from slow dense dull green pigmented mycelium.

Figure 3.4. Paired (somatically compatible) isolates of Hypoxylon multiforme displaying a fan-shaped sector in which growth (linear and aerial) appears to be restricted.


3.3

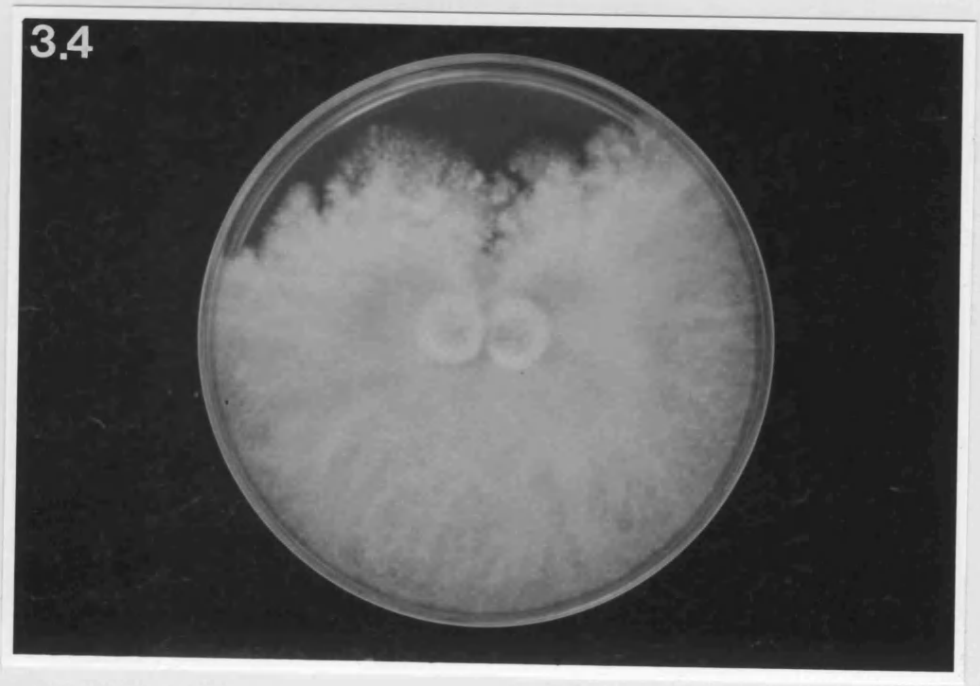

3.4

Figure 3.5. Paired (somatically compatible) isolates of Hypoxylon fragiforme - above (A) and below (B). Note the restricted growth of the mycelium of the colonies on the bottom row (R mycelial type) compared to the "typical" H. fragiforme colonies on the top row in which mycelial growth is unrestricted (U mycelial type).

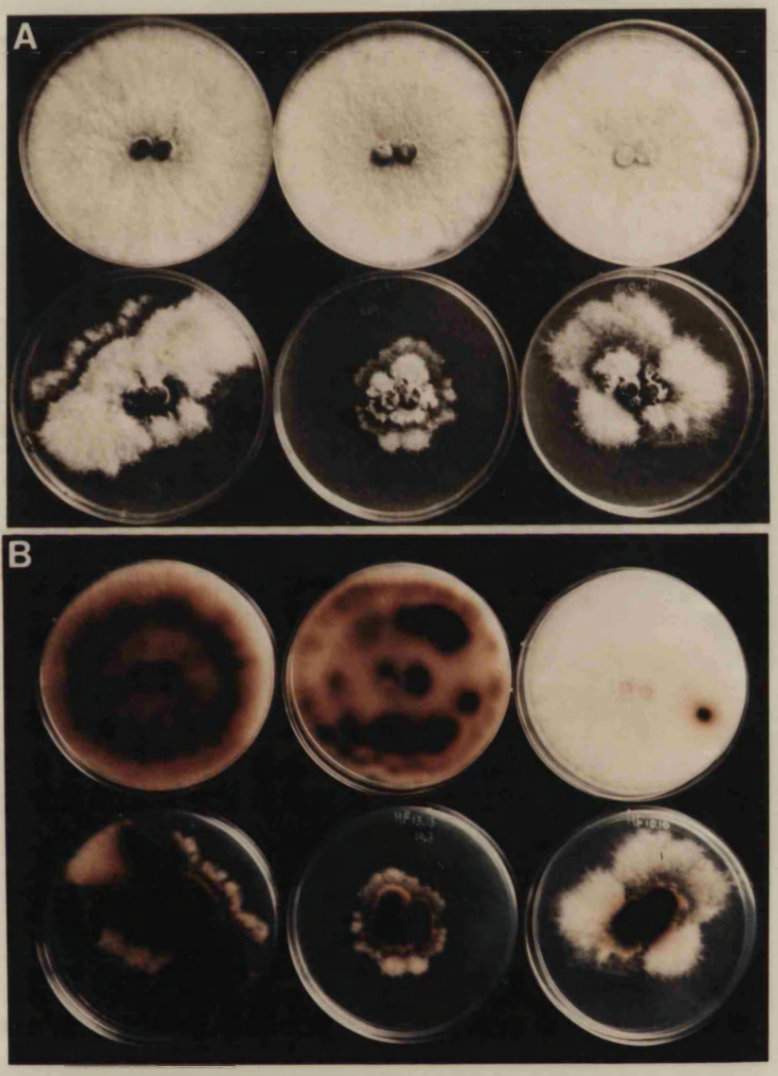
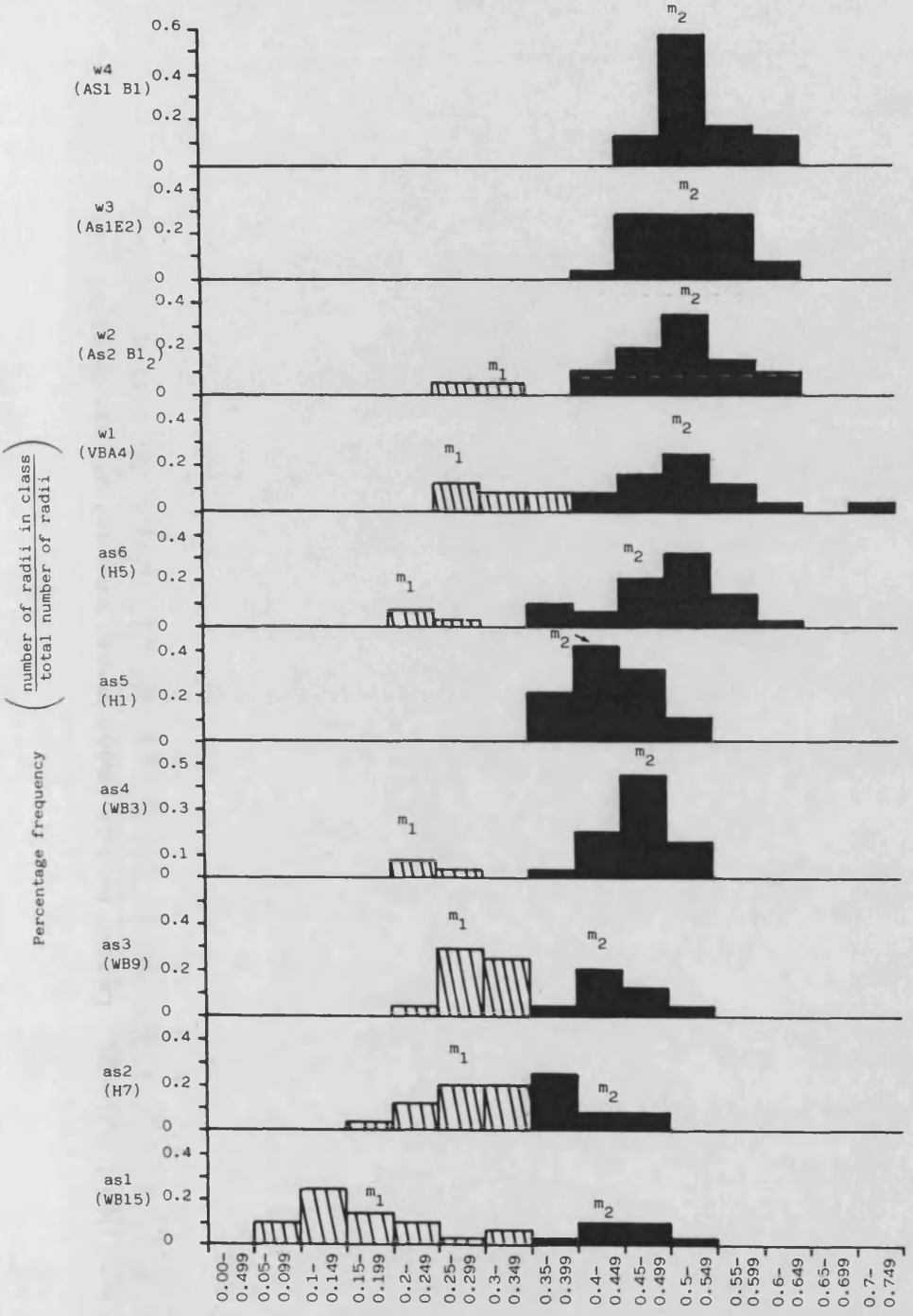

A
B

Figure 3.6. Frequency histogram of radial extension rates of restricted (R) and unrestricted (U) sectors in strains of Hypoxylon fragiforme derived from single ascospores (as) and wood (w).



R

U

according to appearance of mycelium (see text)

m_1 mean of restricted radii

m_2 mean of unrestricted radii

This data was collected from six replicate plates per strain (i.e. a total of 24 radii each).

Figure 3.7. Hypoxylon fragiforme. Leader hyphae (mean measurements) of restricted(R) and unrestricted(U) mycelial types of strains derived from single ascospores(as) and wood(w).

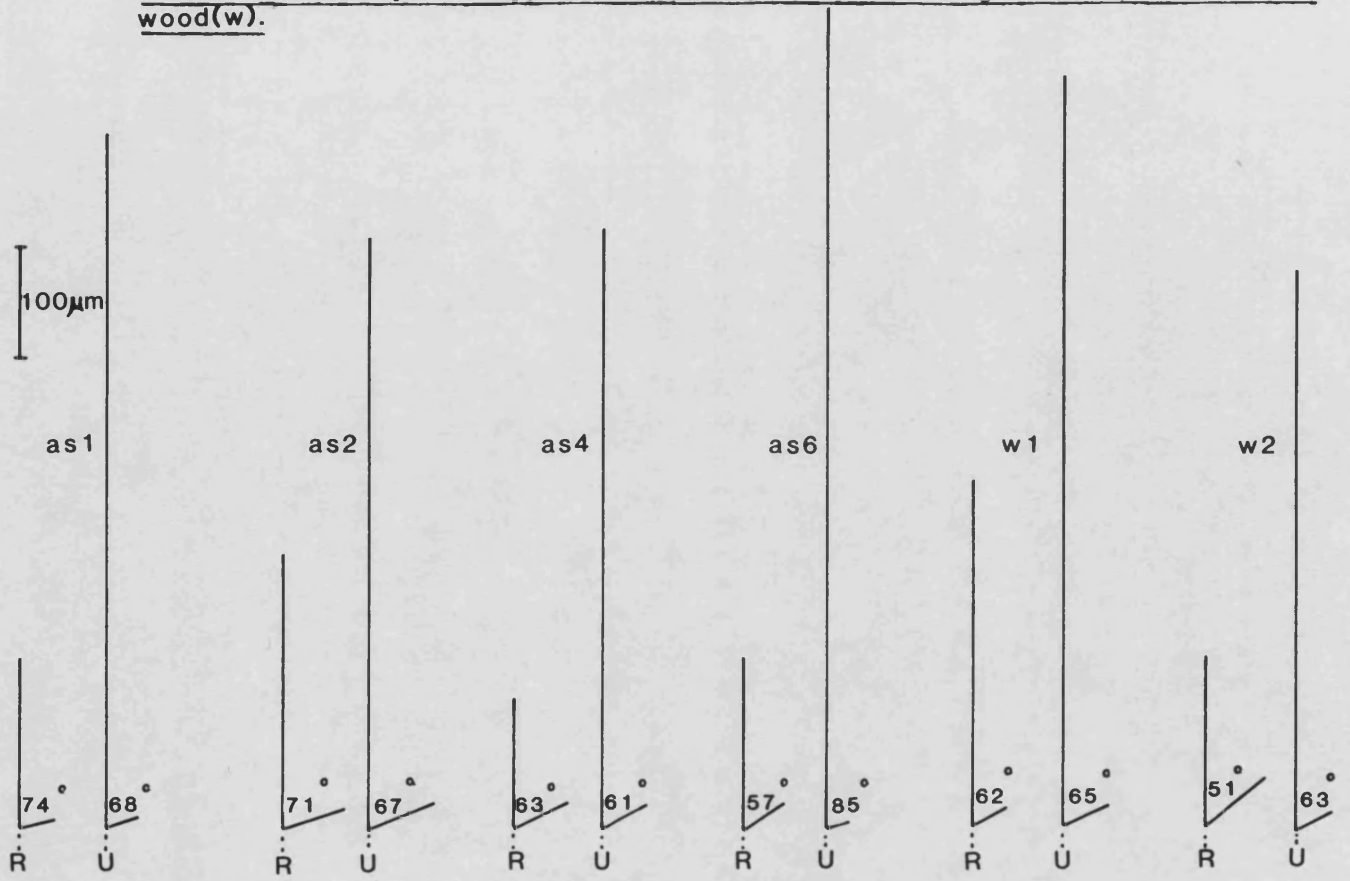


Figure 3.8. Hypoxylon multiforme. Leader hyphae (mean measurements) of restricted(R) and unrestricted(U) colony sectors of strains 1–7 derived from single ascospores.

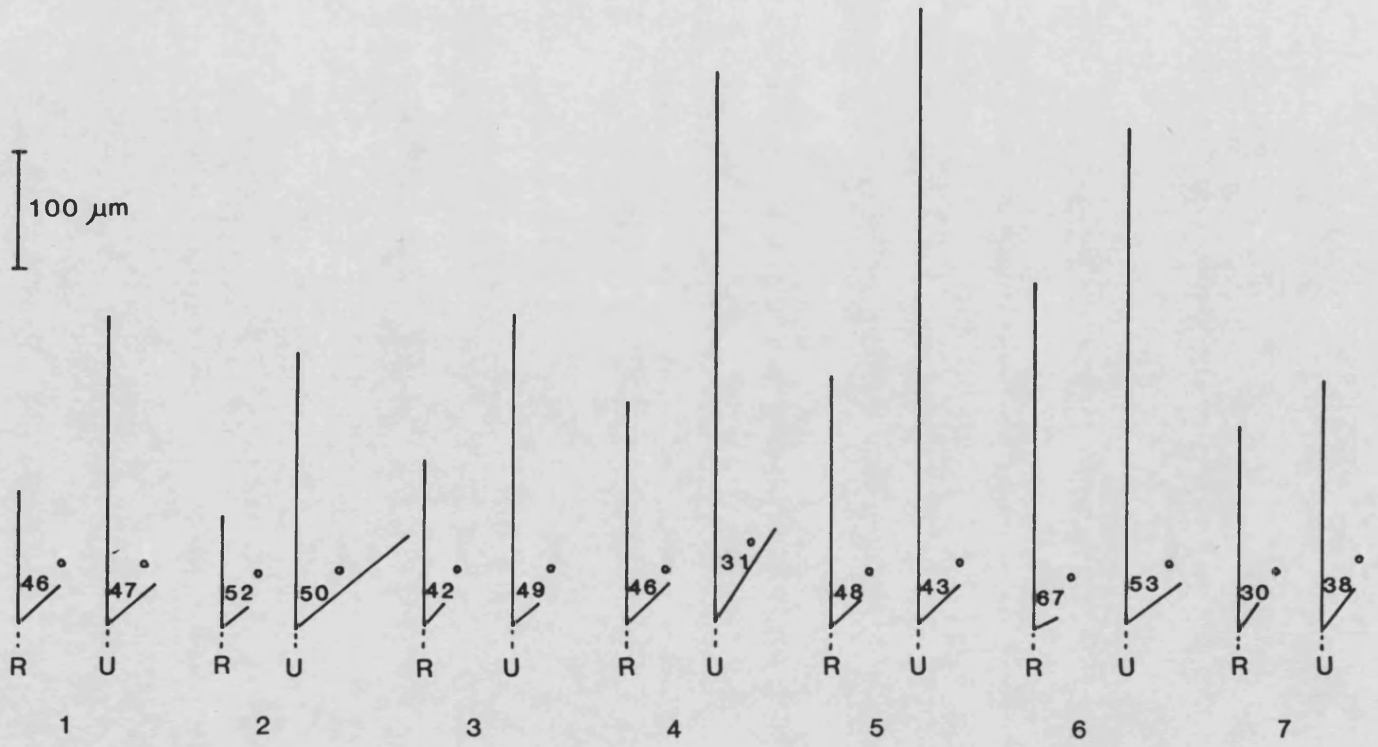


Figure 3.9. "Hypoxylon purpureum". Pigmented hyphae producing prolific abnormal lateral branches in terminal and intercalary positions .

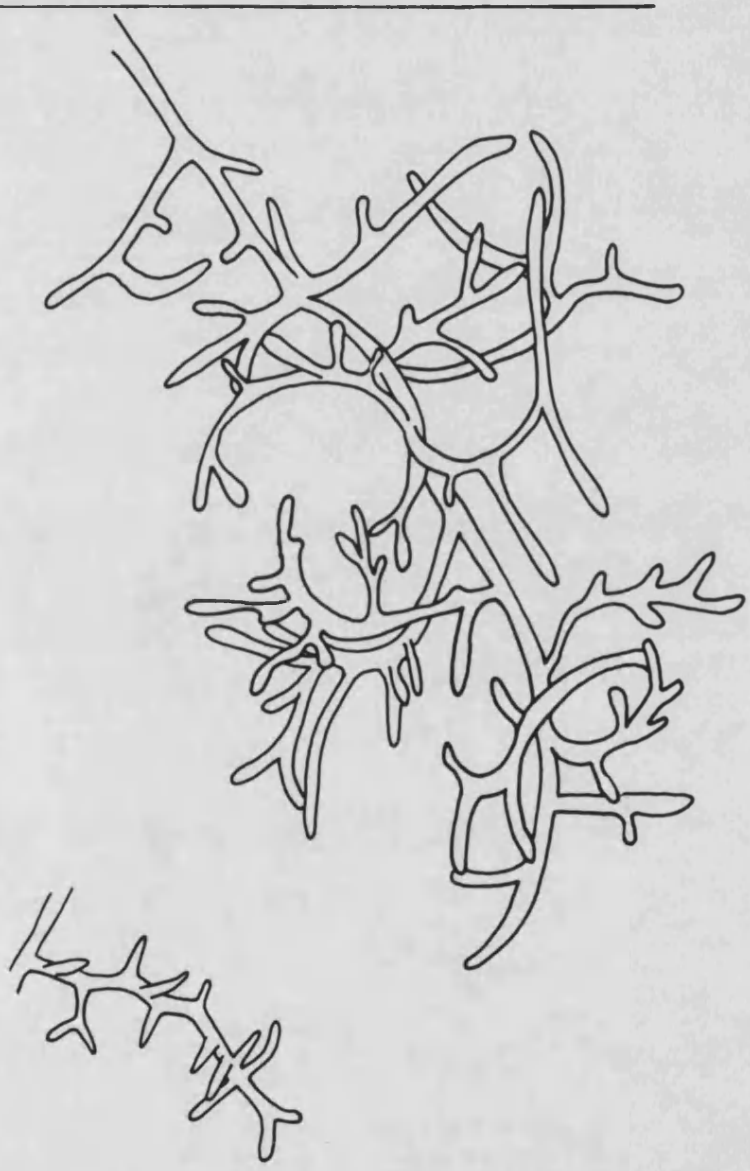


Figure 3.10. Concentrically zoned (CZ) colony of "Hypoxylon purpureum" composed of bands of varying widths (2-9 mm) of different mycelial types. In this colony from the centre to the margin the mycelial types are buff conidial (BC) - **1**, white downy to cottony (WDC) - **2**, dull green (DG) - **3**, buff conidial raised into mounds (MBC) - **4**, DG - **5**, WDC raised into mounds (MWDC) - **6**, DG - **7** and white appressed (WA) - **8**.

Figure 3.11. Conidial isolate ($H8_5$) of "Hypoxylon purpureum" which initially produced a "typical" (T) colony of dull green (**DG**) mycelium but later developed a broad ($\geq$ 10 mm) white downy cottony (**WDC**) band of mycelium around the margin.


3.1 0
1
2
3
4
5
6
7
8

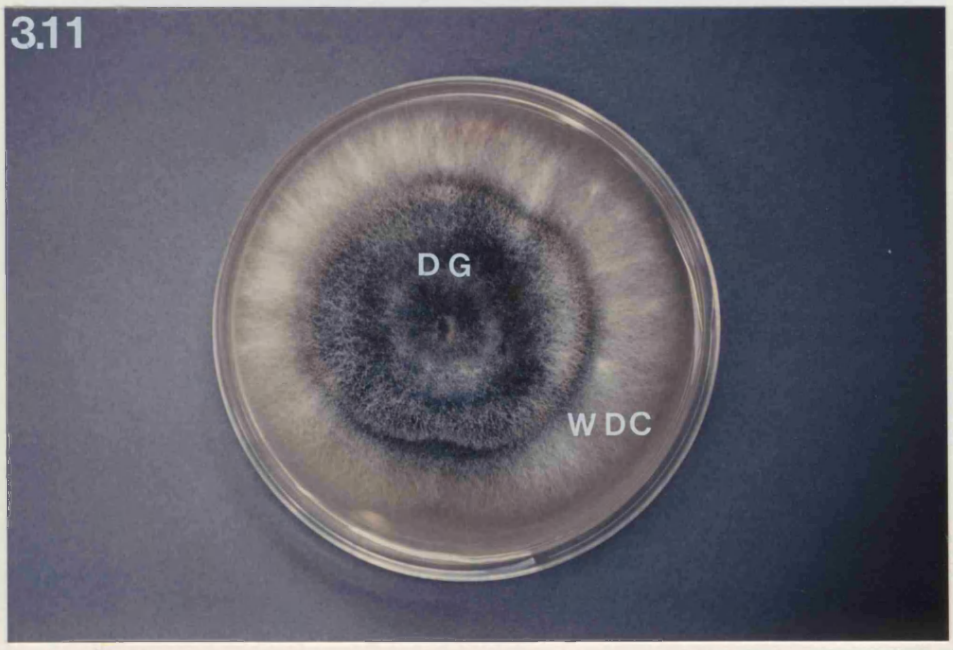

3.11
D G
W DC

Figure 3.12. White felty/white silky (wf/ws) colonies of Hypoxylon serpens arising from a white silky (ws) single ascospore isolate which was subcultured after prolonged incubation (> 90 d). (A) Colony form after 12 d incubation. Note spherical white felty (**wf**) colony from which **ws** mycelial fans are escaping. (B) Colony form after 28 d incubation. The central area of **wf** mycelium has become covered in smoke grey conidial patches.


A
ws
wf

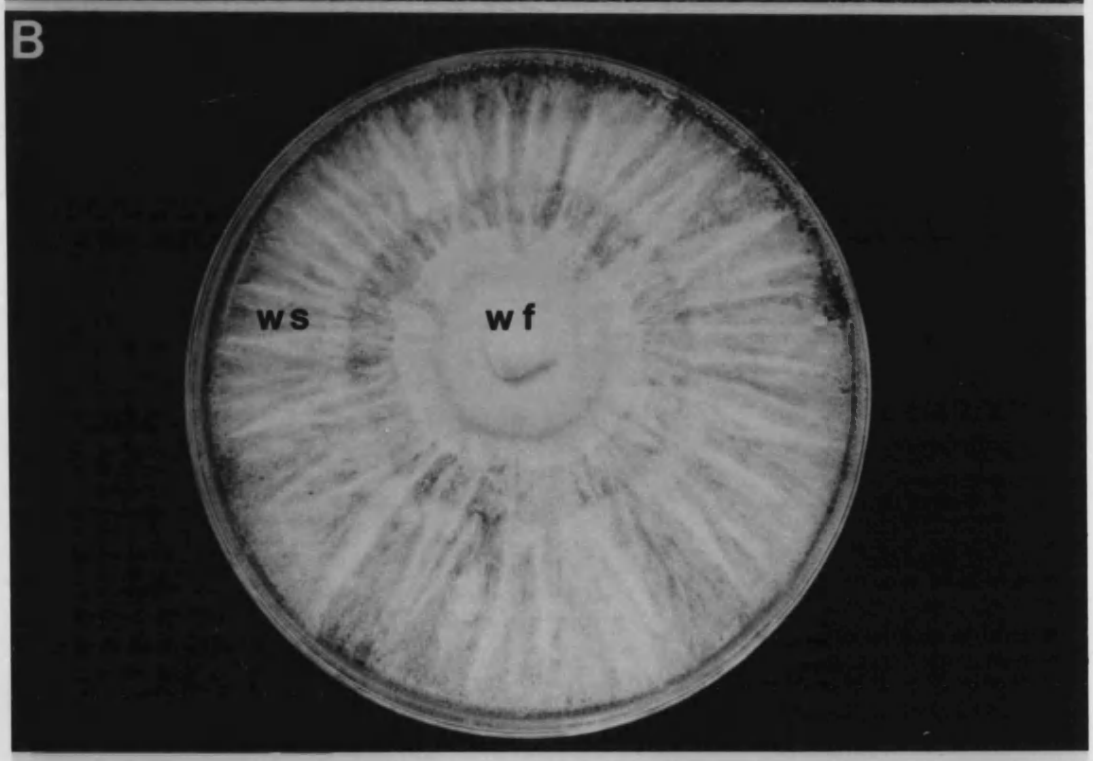

B
ws
wf

Figure 3.13. Hypoxylon serpens. Leader hyphae (mean measurements) of white silky(ws), white felty (wf) and grey conidial (gc) mycelial types.

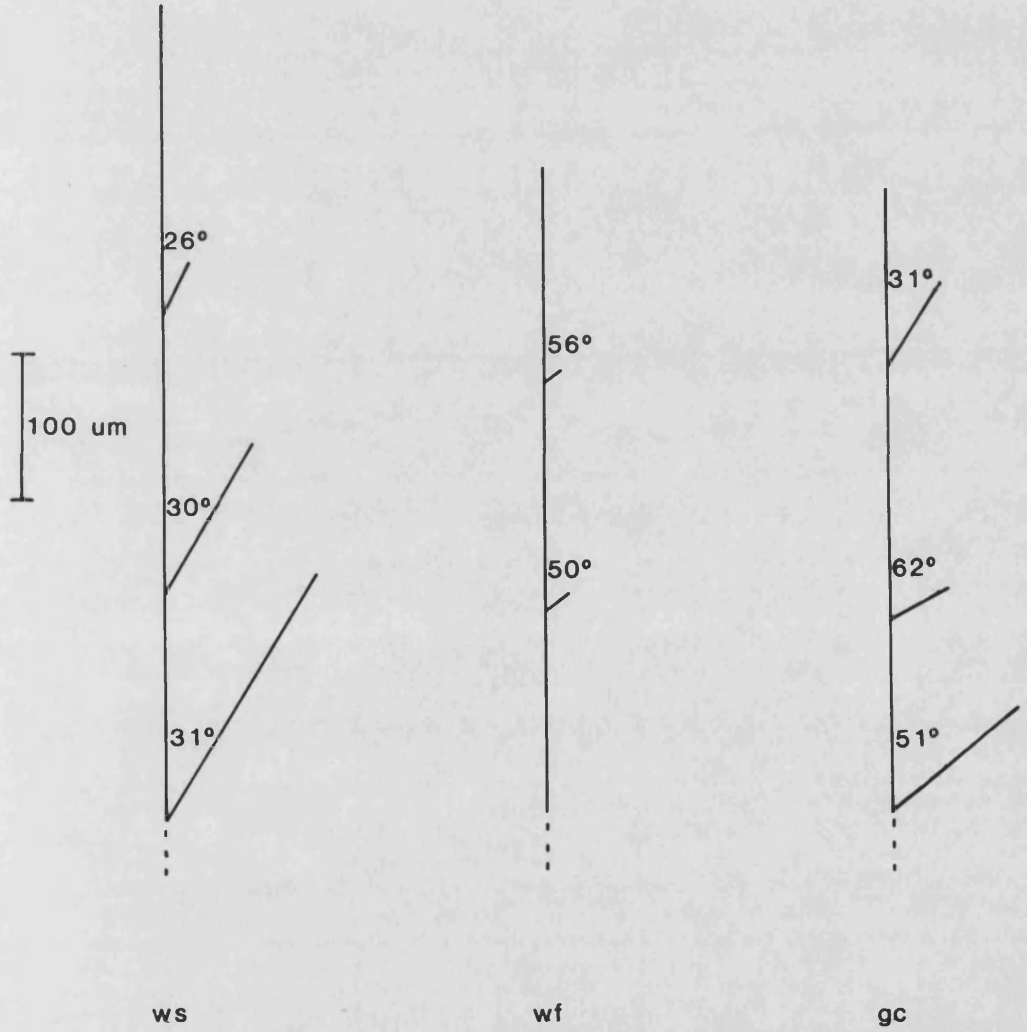
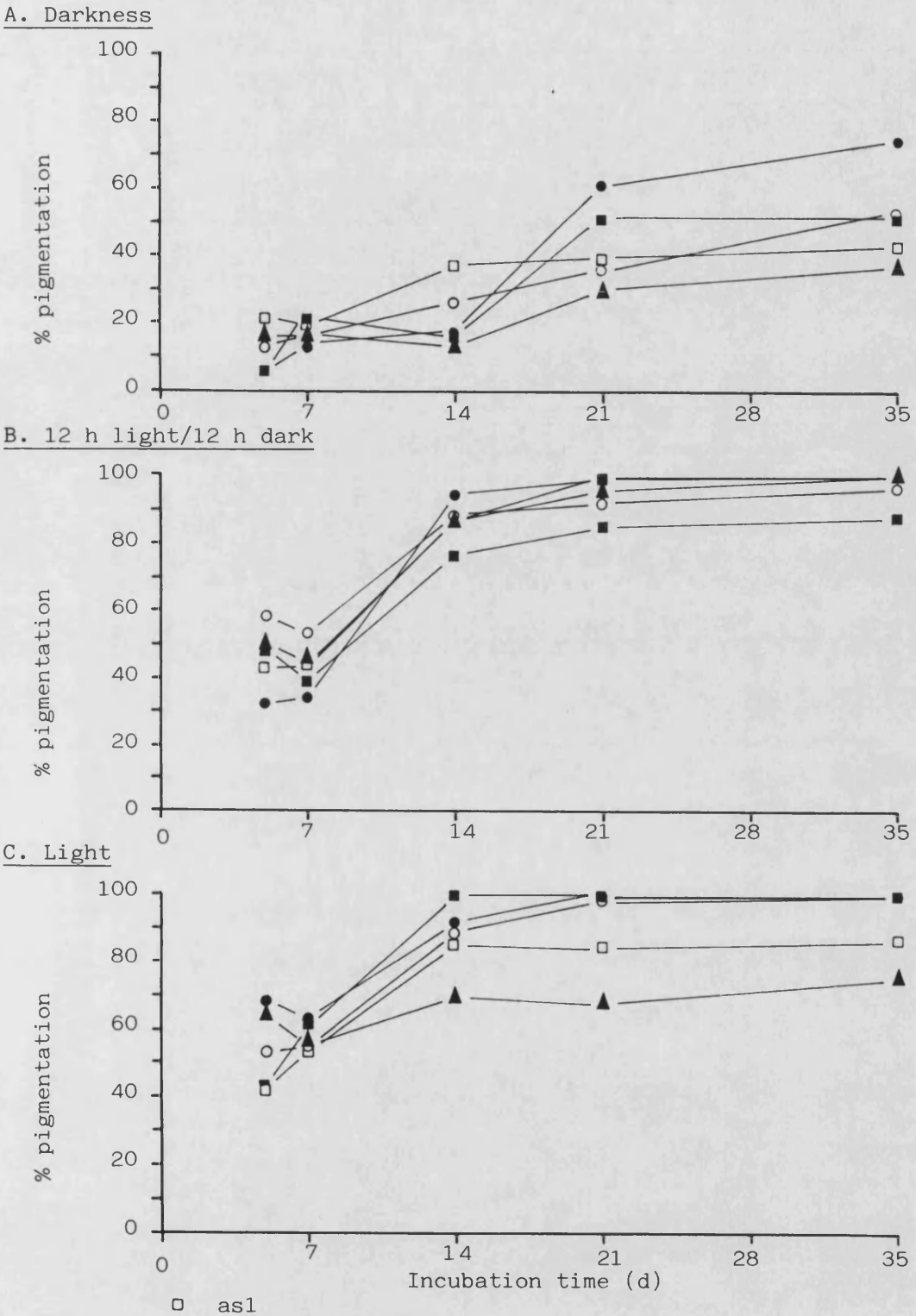


Figure 3.14. Hypoxylon fragiforme. Development of pigmentation (expressed as a percentage of colony size) in single ascospore (as) and wood (w) derived strains incubated at 20°C in darkness (A), 12 h light/12 h dark (B) and light (C).



$$\% \text{ pigmentation} = \left(\frac{\text{diameter of pigmented area}}{\text{diameter of colony}} \times 100 \right)$$

Figure 3.15. Hypoxylon fragiforme. Development of pigmentation (expressed as a percentage of colony size) in colonies of a single ascospore-derived strain (as3) after exposure to different periods of light.

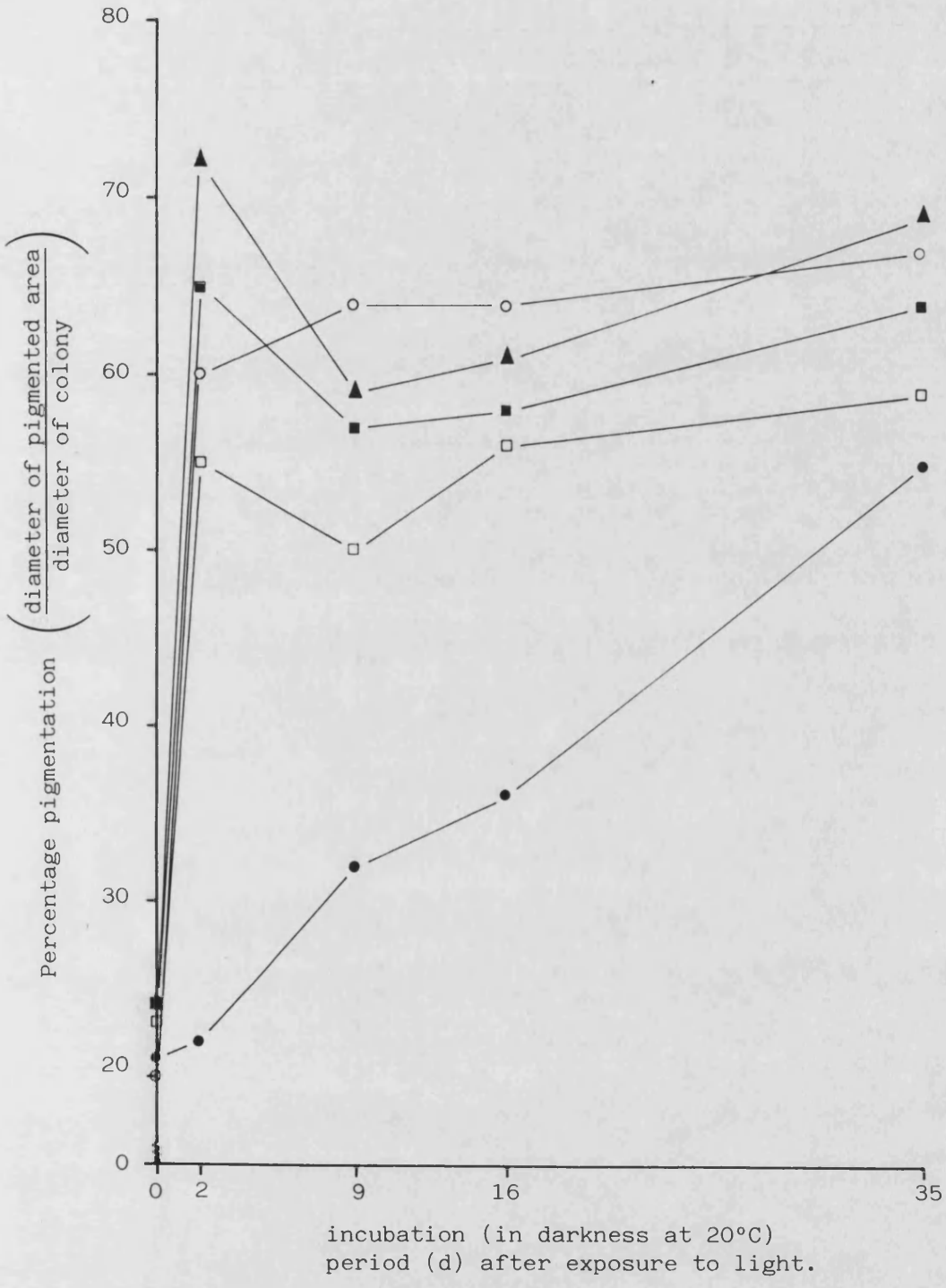
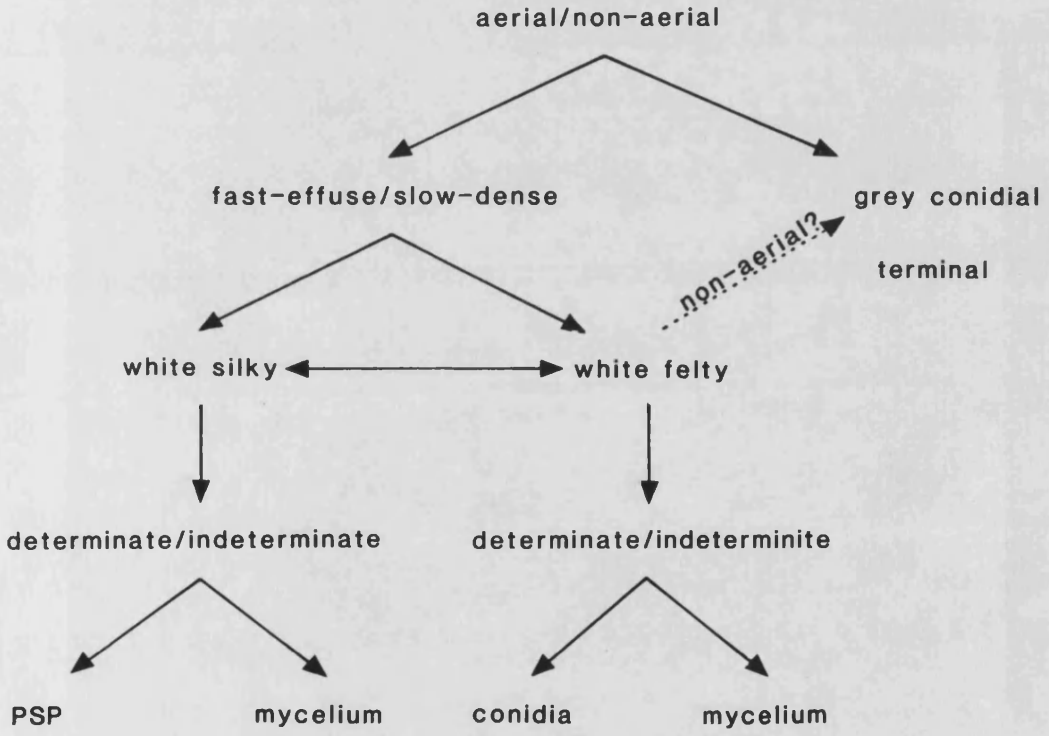


Figure 3.16. Colony of Daldinia concentrica tested for phenol oxidase activity. Wells 1-6 contain dihydroxyphenylalanine, salicylic acid, quinol, catechol, guaiacol and ferulic acid respectively. Note rose colour in the agar around the quinol well (3) indicating laccase activity.

Figure 3.17. Possible distinctive morphogenetic options available to the mycelium of Hypoxylon serpens to account for the observed abrupt alterations in mycelial morphogenesis between white silky, white felty and grey conidial mycelial types.



PSP denotes pseudosclerotial plate.

CHAPTER 4

INTRASPECIFIC INTERACTIONS

4.1 Introduction

As mentioned in Chapter 1 (Section 1.1, ii(a)) a useful basis for identification of genetically and physiologically distinct individuals (genotypes) in fungal populations in nature has been found to be a self-non-self recognition mechanism referred to as somatic incompatibility (Rayner and Todd, 1979; Todd and Rayner, 1980). Adjacent mycelia are said to be somatically incompatible if they form morphologically distinct demarcation zones along their interfaces when they interact together. This will occur if they differ genetically at their polygenic or multiallelic somatic incompatibility loci (Rayner et al., 1984; Rayner and Boddy, 1986). The form of the demarcation zone can vary considerably, both within and between species (Rayner et al., 1984).

The present investigation concerns the interaction patterns between different strains of some xylariaceous species, with a view to determining the usefulness of somatic incompatibility in distinguishing between different genotypes of these fungi. Not only were demarcation zones found to be a reliable indicator of genetic difference, but the form of these zones resembled some of those associated with mating in outcrossing Basidiomycotina. The significance of these similarities is discussed.

4.2 Materials and Methods

These were as described in Chapter 2, Sections 2.1 to 2.6 inclusive.

The development of pseudosclerotial plates (PSPs) produced between interacting mycelia of Hypoxylon serpens (white silky - ws - mycelia) and Hypoxylon mammatum was followed macroscopically and microscopically. Two methods described by Lopez-Real (1975) were used to prepare material for microscopic examination. In one, portions of agar plus mycelium producing PSPs were placed in 90% ethyl alcohol, in which they were dehydrated for 5 h prior to cutting thin sections with a razor blade, and staining with methylene blue. In the other method, interacting mycelia that produce PSPs were inoculated together onto autoclaved (in distilled water at 115°C for 20 min) cellophane (350 P00, British Cellophane Ltd.*) discs overlying 2% MA in 9 cm Petri dishes and incubated in the usual way. At regular intervals in colony development cellophane strips, approximately 2 x 1 cm, supporting mycelia, were dissected out from interaction zones and stained with methylene blue.

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* The cellophane used for isolation of single hyphal tips (Grade 325 P, 80 mm Cannings Parry Packaging Ltd.) was not suitable for this experiment as it had been treated to make it hydrophobic, making the use of a water-soluble stain impossible. Untreated cellophane such as 350 P00, British Cellophane Ltd. was therefore used.

Interaction zones in some species were tested for phenol oxidase and peroxidase activity using the methods described in Chapter 3, Section 3.2, iv.

4.3 Results

i. Interaction types

Strains that were experimentally paired either intermingled with one another, indicating that they were somatically compatible, or a morphologically distinct zone developed at the interface between them, indicating that they were somatically incompatible.

(a) Somatically compatible

In all species somatically compatible strains intermingled together so that the original zone of contact between colonies disappeared, as the morphology of the mycelial mats became uniform, corresponding with the morphology of unpaired strains (Figure 4.1 A,B).

(b) Somatically incompatible

Somatically incompatible reactions could be assigned to two distinct groups - those involving a zone of aerial mycelium and those in which a trough or barrage of sparse mycelium separated colonies.

Aerial mycelium interactions

The majority of the species studied produced interactions belonging to this group. These were marked by the development of

woolly textured aerial mycelium (am) in the interaction zone, containing hyphae which were straighter and wider than normal and had acute-angled branches. Five interaction types were recognized:

Narrow line (Figure 4.1 C,D). A narrow (for width see Table 4.1) line of demarcation was discernible between the colonies. In "strong" interactions this contained an am and was pigmented underneath. In "weak" interactions neither am nor pigment were present.

Wide band (Figure 4.1 E,F). A wide (for width see Table 4.1) zone of more or less constant width developed between the colonies. The zone contained an am and was bounded underneath by pigmented regions.

Bow-tie (Figure 4.2 A-C). Bow-tie shaped demarcation zones containing am and widening outwards from the centre developed between the colonies. At early stages of development of these interactions, when growth covered approximately half the diameter of the plate, a narrow zone of appressed sparse mycelium separated the colonies. Subsequently a fan-shaped am grew out from the ends of this narrow zone, associated with restriction of extension of the parent colony margins, which contained narrow, densely and obtusely branched hyphae with short compartments as well as numerous hyphal knots and coils. As a result, the am widened either symmetrically or asymmetrically as incubation proceeded, and with

time they usually became delimited at their junction with the parent colonies by pigmented zones.

Pincer (Figure 4.2 D-F). These were an apparent variant of bow-tie interactions in which only one of the parent colony margins was suppressed, resulting in the unilateral outgrowth of am. With time the am tended to develop a morphology similar to that of the non-suppressed colony, which consequently encircled the suppressed colony in a pincer-like movement. Unlike symmetric or asymmetric bow-ties, pincer reactions were fully delimited by formation of pigmented zones at only one side of the pairing, next to the suppressed colony.

Hour-glass (Figure 4.2 G-H). Here symmetric or asymmetric am, delimited by pigmented zones, were produced which looked like bow-ties except that they were constricted both at their centre and at their periphery. The reason for the difference in shape from bow-ties appeared to be that the parent colony margins were not, or were only temporarily, suppressed.

Daldinia concentrica, Hypoxylon fragiforme and Hypoxylon nummularium produced all these interaction types while other species developed only some of them (Table 4.2). Certain features, such as zone width in narrow line and wide band interactions, and pigmentation varied between species as shown in Table 4.1. Some strains of H. fragiforme grew as the R mycelial type making it impossible to assess interactions accurately and pairings where

this occurred were recorded as "restricted". The ws mycelial type of H. serpens produced narrow line and wide band and in addition two further interactions:

Ridge (Figure 4.3 A,B). A ridge (at least 3 mm high and 5 mm wide) of white am developed between the colonies and this was marked underneath by one or two pseudosclerotial plates (PSPs). Sometimes after prolonged incubation (> 38 d) the mycelial ridge began to spread unilaterally, asymmetrically or symmetrically into one or both strains respectively.

Cord-penetration (Figure 4.3C,D). This appeared to be a variation of the ridge interaction described above. A ridge of mycelium separated the colonies, but from it, perpendicular to it, white hyphal aggregations or cords extended into one or other of the colonies. Usually cords were produced only in one direction along the entire length of the interaction zone, but occasionally they extended into one colony in one part of the zone, and into the other colony at another part. Very often a PSP was produced along the edge of the interaction zone by the colony that was not penetrated by cords, although occasionally the other colony laid one down or both colonies produced them. The net result of cord-penetration interactions appeared to be the same as a ridge that had migrated unilaterally into one strain rather than the other.

Trough or barrage interactions

Interactions belonging to this group developed in Hypoxylon

fuscum and Hypoxlyon rubiginosum and they were classified into three types, trough (produced by both species), lens (H. fuscum only) and raised mycelium (H. rubiginosum only). Lens and raised mycelium interactions appeared to be variants of one another, as in both a powdery textured mycelium developed after prolonged incubation (> 55 d) from typical trough reactions. Details of the interactions are described below.

Trough (Figure 4.4 A,B). A trough (< 2 mm) of sparse mycelium separated the colonies. Underneath the trough was pigmented.

Pigmentation in H. fuscum varied from one interaction to another and colours included rose, cinnamon, isabelline, luteus or dull green to citrine green. By contrast in H. rubiginosum there was little variation so that troughs were pigmented isabelline or sepia.

Lens (Figure 4.4 C). An oval or lens-shaped area of powdery vinaceous buff to fawn mycelium existed between the colonies usually extending more or less along the line of contact between them. Sometimes the extent of the powdery mycelium was restricted to the area adjacent to the inocula, or occasionally to the periphery of the interaction zone, the remainder being typical of a trough reaction. Underneath, the lens-shaped area was usually marked by greenish olivaceous pigment. Lens reactions developed initially from a buff mycelium produced in the trough that

separated colonies, and this became vinaceous buff to fawn and widened symmetrically or asymmetrically.

Raised mycelium (Figure 4.4 D). A pale luteus, white or olivaceous buff to pale luteus, powdery mycelium, that was raised by at least 1 mm above the adjacent colonies, developed in the hollow of the trough reaction. This mycelium was sometimes only apparent at the distal portions of the interaction line.

ii. Distribution of interaction types in experimental pairings

The pattern of occurrence of different interaction types in experimental pairings for each species is shown in Tables 4.3 to 4.15.

(a) Self pairings

All self pairings in each species were recorded as intermingling.

(b) Non-self pairings

Differences in the frequencies of interactions between non-self pairing types were analysed using the G test for replicated goodness of fit tests (Sokal and Rohlf, 1981 a, b) and are explained below for species producing aerial mycelium interactions and trough or barrage interactions.

Aerial mycelium interactions

In pairings between strains derived from single ascospores from the same perithecium or perithecial stroma of all species, except Hypoxylon multiforme, "Hypoxylon purpureum" and Rosellinia desmazieresii (see below), intermingling was observed only occasionally (Tables 4.3 to 4.9). Interactions with a large amount of am (bow-tie, hour-glass, wide band or ridge depending on the species) were predominant. On the other hand, large am reactions were rare (or narrower) between strains from different sources where narrow line and wide band reactions predominated, narrow line reactions being more frequent between strains from different geographic locations. In species that produced them, the number of pincer reactions did not appear to differ appreciably between pairing types (the wood and monospore isolate pairings in H. mammatum (Table 2.1) that yielded all pincer reactions are disregarded as only one wood isolate was involved).

It appeared therefore that with increasing geographical distance between strains, the amount of am in the interaction zone was reduced, corresponding to a trend of increasing dissimilarity as indicated by the values of G (Table 4.16) which became greater with increasing distance between strains. It should be pointed out, however, that particularly for D. concentrica and H. fragiforme, different interactions were sometimes seen when experiments were repeated, perhaps because of changes in colony development patterns during laboratory culture. It may be best, therefore, not to regard differences between interaction types as absolute distinctions.

Hypoxylon multiforme strains fell into two groups. In the larger group intermingling was recorded in all combinations of ascospore-derived strains from within and between perithecia on the same stroma, and in pairings of ascospore and wood-derived strains from the same decay column of a log (Table 4.10). In the smaller group, comprising two samples, intermingling was only recorded in less than one third of pairings between ascospore-derived strains from the same perithecium (Table 4.11). Paired strains belonging to the larger group mainly resulted in wide band interactions when they were from different sources. An exception occurred in pairings between wood-derived strains from different logs at the same site, which yielded more bow-ties than wide bands. However the significance of this prevalence of bow-ties is uncertain since only a relatively low number of pairings were tested. The wide band reaction was also the principal sort of interaction observed for intraperithecial pairings in the smaller group of H. multiforme strains from sample SW1 and log AA (perithecia a and c), although some bow-ties were recorded. Likewise interperithecial pairings yielded primarily wide bands, although paired wood and monospore-derived strains from the same decay column of a log produced bow-ties and when they were from different decay columns, wide bands and bow-ties occurred in approximately equal proportions.

As for most H. multiforme samples, intermingling was recorded in "H. purpureum" (Table 4.12) and R. desmazieresii (Table 4.13) between all strains derived from the same perithecium, tree or ring respectively. Strains of "H. purpureum" from different sources,

that is separate trees within and between sites, all yielded narrow band reactions. By contrast R. desmazieresii strains from different rings mainly produced wide band reactions when they were derived from ascospores, but wide band and bow-ties in approximately equal frequency when they were from wood and ascospores.

Trough or barrage interactions

A change in the distribution of interaction types with increased genetic difference between paired strains, similar to that described above for aerial mycelium interactions occurred in H. fuscum, but not in H. rubiginosum. Intermingling was not recorded in any non-self pairing of H. fuscum and ascospore-derived strains from the same perithecium predominantly produced the symmetric lens (i.e. the reaction which has most mycelium in the interaction zone) as did wood strains from the same site. However, ascospore strains from separate perithecia on the same stroma produced trough and symmetric lens reactions in approximately equal proportions. Strains from different geographic locations mainly resulted in troughs. Thus there seemed to be a trend from a high frequency of symmetric lens and lower frequency of trough interactions between strains derived from sources that were physically close to one another, to the converse situation (a high frequency of trough and lower frequency of symmetric lens) between geographically separated strains. Asymmetric lens reactions were the least frequently recorded non-intermingling interaction in each of the pairing types. They were recorded in a low number of intraperithecial and interperithecial pairings, but increased in

frequency in pairings between wood strains derived from different locations in the same site and between strains from different sites they occurred in almost 20% of pairings.

In H. rubiginosum (Table 4.15) intermingling was only observed occasionally between ascospores from the same perithecium and trough reactions accounted for at least 93% of interactions in each non-self pairing type. The raised mycelium reaction was recorded between ascospores from the same perithecium and between wood strains from the same site (i.e. strains that were not separated by a great distance), but in both instances its frequency was low compared with troughs.

iii. Genetic designation of interaction zone mycelia

Colonies arising from hyphal tips obtained from the interaction zone in intraspecific pairings of D. concentrica, Hypoxylon nummularium, H. fragiforme, H. serpens, H. multiforme and H. fuscum normally grew out uniformly and intermingled with one of the two types originally paired, whilst producing a reaction zone against the other (Figure 4.5 A). Hence they could be assigned genotypically to one or other of the originals. Sometimes, however, the colonies either grew out uniformly, but interacted against both original types (Figure 4.5 B) or they produced an am which sectored into the two original types (Figure 4.5 C). Genotype assignment, based on these reactions, of tip cultures obtained from a representative set of pairings between ascospore strains from the same perithecium is shown in Tables 4.17 to 4.22.

In D. concentrica, H. fragiforme, H. nummularium and H. serpens the genotype recovered varied with time and position of isolation, except in pincer and cord-penetration reactions where the dominant (or "non-penetrated" in H. serpens) genotype was invariably the one recovered after prolonged incubation from the outer parts of interaction zones. In H. multiforme, genotypes recovered from hyphal tips from bow-tie areas either side of the relic central trough (after 43 d incubation) were always the same as the original type on that side, and one genotype, never both, was obtained from tips taken from the trough itself. By contrast, in H. fuscum, two symmetric lens interactions out of the six that were tested following prolonged incubation (166 d) yielded hyphal tip colonies that sectored into both of the parental types. Only one of the original genotypes, however, was recovered from trough and asymmetric lens interactions, and in the latter the genotype recovered was that of the least or non-penetrated strain. Hyphal tip colonies derived from all H. fuscum interaction types were consistently the same genotype(s) irrespective of where they originated from in the interaction zone (i.e. between the inocula or 2 cm away).

In other species the genetic composition of the interaction zone mycelium was not investigated using the hyphal tip method, but preliminary tests taking small mycelial plugs from either side and within the interaction zone and testing against the original colonies produced results equivalent to those described above. For example in the grey conidial (gc) mycelial type of H. serpens

subcultures from the interaction zones of narrow line, wide band and bow-tie reactions usually produced one of the original genotypes or sometimes broke down into sectors of different parental types. However, subcultures from the mycelial fans of pincer reactions always yielded the spatially dominant genotype.

Conidia from interaction zones of H. mammatum and Rosellinia mammiformis germinated to produce uniform colonies that intermingled with one and produced a reaction zone with the other parental type. Unlike some hyphal tip colonies they did not sector or react with both originals, although in a sample of ten single conidial colonies from each interaction zone there were usually some colonies of one original genotype and some of the other (Table 4.23). Only twice in H. mammatum and once in R. mammiformis were all ten conidial colonies of one original type and in one of the H. mammatum cases it was the spatially dominant genotype in a pincer reaction.

iv. Pseudosclerotial plates in Hypoxylon serpens and Hypoxylon mammatum

Fully developed pseudosclerotial plates (PSPs) formed between interacting mycelia of H. serpens (ws type) and H. mammatum existed in a range of forms which are described below. Microscopic examination of such PSPs revealed that hyphae were tightly packed together and were distinguished from hyphae elsewhere in the colonies by spherical (2 - 5 µm diameter) and tubular (3 - 5 µm wide) swellings which occurred occasionally along their lengths

(Figure 4.6 A, B). Associated with these hyphae was isabelline to chestnut brown pigment.

A detailed study of the development of PSPs in ten pair combinations of different interacting mycelia (pair plates) for each species, demonstrated that the features described above were not apparent until at least 14 d incubation. With respect to H. serpens the first sign of PSP development was a narrow (≤ 1 mm) line of greenish black (two pair plates) or greyish sepia (five pair plates) pigment visible on the underside of colonies. Hyphae in these areas were tangled together in a tight mass with associated isabelline to chestnut brown pigment, and only one hypha was seen to be swollen as described above. These features were absent in the interaction zones of the remaining three pair plates which were unpigmented, but by 21 d an olivaceous grey to greenish black, narrow (≤ 1 mm) line was seen in each one along one side of the interaction zone. By this time the pigment on the underside of all the other pair plates was greenish black and in these regions hyphae with spherical and tubular swellings were abundant. After 49 d incubation, PSPs existed in a variety of forms including a narrow (≤ 1 mm) greenish black line along the centre (three pair plates), or along one (one pair plate) or both sides (two pair plates) of the interaction zone. Alternatively a narrow (< 1 mm) isabelline line along the centre was bounded by lines (1 mm wide) or more typically irregular shaped (2-4 mm wide) bands of greenish black pigment (four pair plates).

In H. mammatum after 7 d incubation, except for two pair plates that were non-pigmented, straw coloured areas containing densely packed hyphae were apparent between interacting mycelia. This also applied to five pair plates at 14 d and the straw pigment in the other five plates had darkened to smoke grey (isabelline microscopically). Although swollen hyphae were absent from these areas, they were present in regions of 21 d old colonies marked underneath by narrow (1 mm) greenish black pigmented lines either in the centre (one pair plate) or along one (four pair plates) or both sides (four pair plates) of the interaction zone. At this stage the interaction zone of one pair plate was still only pigmented straw colour. By 49 d greenish black PSPs were present in all pair plates, but there was considerable variation in form, from a narrow (1 mm) line along one (three pair plates) or both sides (one pair plate) of the interaction zone, or as a discontinuous band of irregular width (1-4 mm) within (one pair plate), or along one (one pair plate) or both sides (three pair plates) of the interaction zone.

v. Phenol oxidase and peroxidase activity of interaction zones

As explained in Chapter 3 (Section 3.3, iv), the development of colour in the agar and mycelium around substrate wells containing phenols was regarded as an indication that the mycelium possessed phenol oxidase, that is laccase (for the phenols salicylic acid, quinol, catechol, guaiacol and ferulic acid) or tyrosinase (for dihydroxyphenylalanine) activity. The results are shown in Table 4.24.

The interaction zones between paired mycelia of H. serpens failed to elicit a reaction with any of the six substrates, as did those of D. concentrica and R. mammiformis. Colour around the quinol well, however, was observed in the interaction zones of H. mammatum, H. multiforme, H. nummularium, "H. purpureum" and H. rubiginosum. Similarly colour was recorded for guaiacol and catechol in the interaction zone between paired strains of H. mammatum, one of which had responded to these substrates when unpaired. In contrast the H. mammatum interaction zone also responded to dihydroxyphenylalanine and interaction zones of H. fragiforme developed colour with this and guaiacol.

The interaction zones of all species tested possessed peroxidase activity (Table 4.24). Although the amount of activity was measured on an arbitrary scale (+ low, ++ moderate and +++ high) some species consistently showed the same level in interaction zones. For example H. nummularium, "H. purpureum" and H. mammatum always had high activity and H. rubiginosum always had low activity. By contrast high and moderate activity was shown by different interaction zones of H. fragiforme and H. serpens, and high and low activity in H. multiforme. Only one interaction zone of D. concentrica was tested and this had a moderate activity.

4.4 Discussion

In accordance with Rayner et al. (1984) and Rayner and Boddy (1986) strains that were somatically compatible (i.e. their mycelia intermingled together when paired) were regarded as having the same

genotype with respect to somatic incompatibility loci. By contrast, strains that were somatically incompatible (i.e. they mycelia formed morphologically distinct demarcation zones along their interfaces when they interacted together) were regarded as different genotypes with respect to somatic incompatibility loci.

Although it was convenient to divide them into broad morphological groups (aerial mycelium, trough or barrages), the non-self (somatically incompatible) reactions recorded above may be considered to represent a spectrum of recognition phenomena resulting in varying degrees of non-self rejection or acceptance. This would accord with ideas described by Rayner et al. (1984) whereby rejection and acceptance are in a delicate balance with one another, which is affected by the degree of genetic difference between self and non-self.

In the present investigation absolute rejection for example may be manifested as a pigmented trough of sparse hyphae, a "no-mans land" into which neither mycelium can penetrate or cross. The greatest degree of acceptance, in which perhaps rejection is overridden (see below) may be the broad zones of am produced in the bow-tie, hour-glass or pincer type reactions. Hyphal tip subculturing showed such interaction zones could contain a temporary heterokaryon as a result of nuclei from one mycelium (donor nuclei) being allowed access into the other (an acceptor mycelium). The extent of this heterokaryon appeared to be limited as it was eventually bounded by pigmented rejection zones.

Between the two extremes of the spectrum lie the other interaction types, their position being determined by the net balance between rejection and acceptance. Hence the narrow line and wide band reactions may be expressions of considerably restricted and partial acceptance respectively, whilst the regeneration of a temporarily heterokaryotic mycelium in troughs resulting in lens interactions of Hypoxylon fuscum, perhaps represents a degree of acceptance following prolonged rejection. This situation may be equivalent to the process of override proposed by Rayner et al. (1984) as a way in which somatic incompatibility (rejection) between mating compatible homokaryons of outcrossing Basidiomycotina is overcome, allowing nuclear migration and association of complementary nuclei to form a stable heterokaryon.

The bow-tie and wide bands of Hypoxylon multiforme that were bisected by troughs are perhaps equivalent to the lens interactions of H. fuscum in that they may represent acceptance following prolonged rejection. Heterokaryosis was not demonstrated in this species perhaps because hyphal tips were only isolated following prolonged incubation, by which time the heterokaryon may have broken down. The ridge interaction of white silky (ws) Hypoxylon serpens was previously recorded for this species by Dowson (1982). Although different in shape from bow-tie, hour-glass and pincer types (possibly because the mycelium was silky rather than woolly textured like the other xylariaceous species) it was shown to be temporarily heterokaryotic. Hence it may be considered as a different expression of nuclear access resulting from the same

underlying mechanism. The cord-penetration reaction, however, appears to be different, an increase in territory being achieved by the ability of the mycelium to alter morphologically, producing cords not unlike those produced by certain Basidiomycotina in culture (Rayner and Webber, 1984).

This morphological alteration at the interface between interacting mycelia of different genotypes, accords with evidence that recognition phenomena appear to be able to regulate developmental switching of mycelia between morphologically and functionally distinctive states or modes (Rayner and Coates, 1987; as mentioned in Chapter 3, Section 3.1). Indeed, here in the Xylariaceae non-self rejection seemed to involve activation of senescence pathways, resulting in appressed growth and pigmentation. In addition, ws H. serpens and Hypoxylon mammatum rejections produced a mode switch (a diffuse/compacted dimorphism) to pseudosclerotial plate (PSP) formation.

Three distinct phases in PSP development were established in the basidiomycetes Armillaria mellea and Stereum hirsutum (Lopez-Real, 1975). These were hyphal proliferation, followed by hyphal swelling and aggregation, and pigmentation. However, only the latter two were observed in Hypoxylon species, and they appeared to occur together. The proposal that damage to the mycelium may be the initial stimulus for PSP formation (Lopez-Real and Swift, 1977) would accord with their development in interaction zones, where recognition responses may harm one or both mycelia. However, it

should be pointed out that ws H. serpens produced PSPs spontaneously in unpaired mycelia (see Chapter 3, Table 3.1) indicating that other factors may be involved in PSP development.

An increase in phenol oxidase activity in the interaction zone is also associated with rejection reactions (Rayner and Coates, 1987). However there was no evidence for this here, except for Hypoxylon fragiforme and H. mammatum (interaction zones had laccase and tyrosinase activity, whilst unpaired mycelia only possessed the former). Peroxidase activity did however appear to increase in interaction zones of all species tested, except for H. rubiginosum where it decreased. The reason for this decline in activity is not clear.

Non-self acceptance seemed to involve activation of juvenile pathways resulting in abundant production of am. Apparently associated with this, in strains producing pincer and bow-tie reactions, was the inhibition of marginal extension of one or both thalli respectively (possibly involving alterations in internode length and branch angle). These mode switches together presumably produced the unilaterally or bilaterally outwardly flared zones of am characteristic of these reactions.

The morphologically distinctive mycelial modes observed in unpaired mycelia of H. fragiforme, H. multiforme (restricted (R) and unrestricted (U) mycelial types), "Hypoxylon purpureum" (typical (T) and concentrically zoned (CZ) colonies) and H. serpens

(white silky (ws), white felty (wf) and grey conidial (gc) mycelial types) recorded in Chapter 3 (Section 3.3, ii, (a)-(d)) did not appear to be specifically triggered by recognition phenomena. Even in H. fragiforme interactions, where mycelia frequently adopted both R and U patterns of development, switching between the two seemed to occur at random - independent of the recognition response.

Somatic incompatibility serves to delimit genetically non-alike individuals, conferring on them a variety of advantages. These include the stabilization and maintenance of favourable gene combinations, independent capture and utilization of resources (resulting in territorial behaviour) and lessened susceptibility to internal pathogens (Rayner et al., 1984). Why it is that these xylariaceous fungi appeared to allow integration (through acceptance of non-self), albeit limited, between somatically incompatible individuals needs to be considered. Three distinctive consequences of non-self acceptance may be significant.

Firstly, non-self acceptance may extend the range over which fertilization can occur, which would otherwise be confined to the junction zone between two strains. Although there is no evidence here for fertilization in the Xylariaceae, this has been shown in other Ascomycotina. For example in Ophiostoma ulmi synnemata and perithecia production was increased the greater the "penetration effect" (i.e. where the mycelium of one strain penetrates or invades that of another). It was not clear whether this effect was

a result of hyphal interdigitation or from nuclear migration, although the former was favoured (Brasier, 1984). Further, in Cochliobolus heterostrophus the existence of a multiallelic system that was independent of the biallelic mating system was demonstrated. This multiallelic system allowed for a degree of non-self acceptance between strains and was associated with an increase in fecundity (Kolmer and Leonard, 1986).

Secondly, non-self acceptance may provide a way in which the domain of a particular genotype is extended. For example in xylariaceous bow-tie, hour-glass or pincer reactions, although the am was temporarily heterokaryotic, containing two types of associated nuclei (as shown by hyphal tip subculturing), ultimately one nucleus type became dominant. Consequently this type made a territorial gain.

A third possible consequence of non-self acceptance is that it may lead to the generation of a new genotype, at least with respect to somatic incompatibility loci. Evidence for this was obtained when a colony arising from a hyphal tip, from an interaction zone, grew out uniformly and produced reaction zones with both strains originally paired (Figure 4.5 B). This indicates that following production of a temporary heterokaryon, in which nuclei from both strains were associated, recombination may have occurred creating a new genotype. This contrasts with the situations where one nucleus type apparently dominated in the am (Figure 4.5 A), and where two nucleus types seemed to be associated in the am as long as the

latter was supported by the original strains, but in their absence broke down into the two types (Figure 4.5 C). The possibly "recombinant" genotype was perhaps homokaryotic as it was morphologically identical to the original homokaryotic strains (derived from single ascospores), whilst differing from the the temporarily heterokaryotic am.

The populations studied appeared to fall into two categories. In the first and largest category were those in which the presence of somatic incompatibility and cultural variability between ascospore progeny from the same perithecium indicated sexual outcrossing. In the second category were those in which such progeny were all culturally similar and somatically compatible (i.e. there was no variation) indicating that they were produced by a non-outcrossing mechanism (H. multiforme, "H. purpureum" and Rosellinia desmazieresii). The role of outcrossing and non-outcrossing systems in the generation and maintenance of population variation, will be considered further in Chapter 5.

In the outcrossing populations intermingling interactions between sib ascospores (described above) were scarce, occurring at a level commensurate with the possibility of obtaining sister spores from the same ascus. This provides evidence that the somatically incompatible interactions in the remaining combinations were under polygenic control. A system with fewer genes would reduce the probability of heterozygosity between loci and therefore possibly allow more strains to intermingle. Bow-tie, hour-glass and

pincer reactions, which in species that formed them were the predominant somatically incompatible reactions between strains from the same perithecium, seemed to be different expressions of the same interaction phenotype (i.e. a lot of non-self acceptance). This may be partly a result of developmental status of the mycelia affecting the outcome. However it also suggests that the recognition factor may be multiallelic and have insertion points at several or numerous loci.

The bow-tie, hour-glass and pincer reactions resembled some reaction zones associated with mating in outcrossing Basidiomycotina. In order to explain this a brief outline of such basidiomycete reactions will follow, according to Rayner et al. (1984).

Outcrossing in Basidiomycotina is dependent on the somatic fusion and establishment of a stable secondary (heterokaryotic) mycelium between genetically different homokaryons. Somatic incompatibility between such homokaryons must be overridden by mating compatibility mechanisms (see Chapter 1, Section 1.1, ii, (a)). A working hypothesis was proposed including three fundamental functions associated with mating compatibility (i.e. secondary mycelium formation). These are access migration, acceptor migration and stabilization. Access migration is the entry of donor (non-self) nuclei into an acceptor mycelium, whilst acceptor migration is the migration of these nuclei through the acceptor. This occurs via pre-existing hyphae through septa, at a rate determined by the acceptor mycelium. Stabilization is the stable association of the

two types of complementary nuclei, donor and acceptor, in a single secondary mycelium. The result of pairing, for example sib homokaryons, will depend on which of these three functions operate, as explained below. It should be pointed out that the precise way in which these three functions are co-ordinated and controlled genetically will vary between species.

If access migration acts alone the outcome is a "bow-tie" reaction. That is a bow-tie shaped zone of appressed hyphae develops between paired mycelia (thought to be where donor nuclei invade acceptor mycelium). Eventually mycelium of one nucleus type regenerates in this area with the net outcome that this type has partially or completely replaced the other and made a territorial gain. The extent of replacement is dependent on whether nuclear migration is unilateral or bilateral. The "bow-tie" reaction was first reported between monospore sib pairings of Stereum hirsutum (Coates, Rayner and Todd, 1981), but reactions with apparently similar characteristics have subsequently been observed in several Basidiomycotina (Coates and Rayner, 1985a). It usually occurs between sib homokaryons with identical mating type loci (i.e. mating type incompatible) which differ at a multiple allelic locus elsewhere.

Where access migration is accompanied by stabilization the outcome is a localized bow-tie shaped, zone of secondary mycelium in which the two nucleus types are stably associated. Alternatively access, acceptor migration and stabilization occur together

producing an unrestricted secondary mycelium across both original homokaryons.

Xylariaceous bow-tie, pincer and hour-glass interactions had three features that were reminiscent of basidiomycete "bow-tie" reactions. These were their shape, inhibition of marginal extension and eventual territorial gain. They also resembled basidiomycete mating reactions that result in localized bow-tie shaped secondary mycelium. This is because they produce a non-sporulating am with straight, wide hyphae and narrow angled branching patterns. Further, they apparently achieve territorial dominance via a secondary mycelial phase, so that the only real difference from a basidiomycete mating reaction lies in the lack of stabilization of the heterokaryon.

Perhaps as a result of mating being scored differently in Ascomycotina and Basidiomycotina, it is commonly assumed that no parallels can be drawn between the mating systems in these two groups. In Ascomycotina mating is scored on the basis of formation of viable ascospores which, in outcrossing species is controlled by a dimictic system (i.e. biallelic genetic locus). This system is independent of recognition responses between somatic hyphae. However, in Basidiomycotina, mating is scored on the basis of the production of a secondary mycelium, following a non-self acceptance response in sexually undifferentiated hyphae. This is controlled by a unifactorial, bifactorial or exceptionally trifactorial multiallelic genetic system. In demonstrating that non-self access

in sexually undifferentiated hyphae occurs in Ascomycotina (as explained above bow-tie, pincer and hour-glass reactions are expressions of it) and that it is regulated by a multiallelic system, the present study points to links with the Basidiomycotina systems. Further, it can be speculated that by combining the multiallelic system controlling non-self access with biallelic control of stabilization in Ascomycotina, a multiallelic unifactorial or bifactorial mating system, such as that which occurs in Basidiomycotina, could arise.

The apparently inverse relationship between the rate of expression of somatic rejection and acceptance (in the form of access of donor nuclei into an acceptor mycelium) is another possible similarity between xylariaceous mycelial interactions and certain Basidiomycotina mating reactions. The relationship is indicated by the reduction of access, manifested by an increased proportion of wide band and narrow line reactions between isolates from different locations, possibly correlating with increased genetic differences. This is a situation that has been demonstrated in the basidiomycete Thanatephorus cucumeris (Anderson, 1984) in which heterokaryotic tufts produced between mating compatible homokaryons may be comparable to am production in xylariaceous species. Between field isolates of T. cucumeris from widely separated geographic areas, only a few small tufts of heterokaryotic hyphae are formed but, when selected homokaryons from the same field isolates are paired, large tufts occur.

In species that produced aerial mycelium interactions the reduction of access between wood strains from the same source (which produced primarily narrow lines and wide bands) compared to ascospore strains (that resulted in a high proportion of bow-ties etc.) may be accounted for by the developmental state of their respective mycelia (Rayner et al., 1984). For example it may be that the juvenile state of mycelia derived from ascospores may allow more acceptance/access as these strains are in an exploratory mode of growth consistent with the capture of resources and increase of their territory. Wood strains by contrast tend towards rejection as they are mature, have established domains and are perhaps in a combative mode, more concerned with holding on to territory which they already possess.

There is evidence that some of the mycelial recognition systems observed here in the Xylariaceae may exist in other Ascomycotina. For example Fatemi and Nelson (1978) established (using a hyphal tip method) that white mycelial tufts between paired field isolates and their monoconidial progeny of Pyricularia oryzae were heterokaryotic. In addition white mycelia occurring between interacting isolates of Ophiostoma ulmi have been described by Brasier (1984). Indeed, it is not clear whether the "penetration effect" (mentioned above and in Chapter 1, Section 1.1, ii, (c)) in this species, is related to the access phenomena described here for the Xylariaceae (Rayner and Boddy, 1986). Further, mycelial interactions like those of xylariaceous species (54% bow-ties, 24% wide bands, 19% narrow lines and 3% intermingling), involving white am and straw coloured

pigment were observed in non-self pairings between single ascospore strains from the same perithecium of a Diatrype species (Sharland and Rayner, unpublished). These studies perhaps indicate therefore that access (acceptance) phenomena/temporary heterokaryosis may be widespread in the Ascomycotina and certainly point to the urgent need to conduct more studies in this direction.

Table 4.1. Features of aerial mycelium interactions that were characteristic for particular species.

Species/mycelial type	Characteristic features					
	Colour of aerial mycelium	Zone width(mm) Narrow line	Zone width(mm) Wide band	Pigmentation (underneath colonies) Initially (7 - 14 d)	Pigmentation (underneath colonies) Darkening to (>21 d)	Others
Daldinia concentrica	white	≤3	>3	amber or luteus	dark mouse grey or fuscous black	In pincer interactions including "felty" and woolly colony types (see Table 3.1) the woolly types was normally dominant.
Hypoxylon fragiforme	white (pistachio green when above dark bluish green pigment)	≤10	>10	dark bluish green or occasionally isabelline in centre, bounded on either side by narrow umber lines		Where restricted (R) and unrestricted (U) mycelial types were paired the R strain was encircled by the U.
Hypoxylon nummularium	white	≤10	>10	herbage green to dark herbage green or, less commonly, amber	sienna, umber and finally sepia	-
Hypoxylon serpens grey conidial (gc)	smoke grey/ grey olivaceous	≤3	>3	pale mouse grey or in 'strong' narrow line interactions a white or cream line (1 mm wide) bounded by pale mouse grey lines (1 mm wide)		Aerial mycelium was conidial as was mycelium of the rest of colonies.
Hypoxylon mammatum	white	≤1	>1 (usually at least 3)	straw or luteus	smoke grey or more usually greenish black	Following a minimum of 28 d incubation conidia were produced in smoke-grey coloured areas either adjacent to, or within, interaction zones.

Table 4.1. (continued).

Species/mycelial type	Characteristic features					
	Colour of aerial mycelium	Zone width (mm) Narrow line	Wide band	Pigmentation (underneath colonies) Initially (7 - 14 d)	Darkening to (>21 d)	Others
Hypoxylon multiforme	white (after prolonged incubation, i.e. > 84 d, primrose to straw)	-	>3	none, except occasionally pale vinaceous to livid vinaceous		Initially both wide band and bow-tie interaction zones were bisected by a central narrow (1 mm) trough of sparse mycelium of lysed hyphae and hyphal ghosts. As incubation proceeded the trough became increasingly diffuse as mycelium regenerated in it.
Hypoxylon serpens white silky (ws)	white	≤1	>1	one or two black or isabelline to sepia pseudosclerotial plates		Other interaction types were ridge and cord-penetration. For details see text.
Rosellinia desmazieresii	white initially (7 d) later (> 14 d) mouse grey	≤1	>1	straw	dark mouse grey occasionally greenish black pseudosclerotial plates	-
Rosellinia mammiformis	white	≤2	-	none	honey to isabelline	In some pairings one or both isolates produced a pale mouse grey line (up to 2 mm wide) of conidia along the interaction zone.
"Hypoxylon purpureum"	white	≤2	-	none		-

Table 4.2. Aerial mycelium interaction types produced by xylariaceous species.

Species/mycelial type	Interaction Types					
	Narrow line	Wide band	Bow-tie	Pincer	Hour-glass	Others
Daldinia concentrica	✓	✓	✓	✓	✓	-
Hypoxylon fragiforme	✓	✓	✓	✓	✓	-
Hypoxylon nummularium	✓	✓	✓	✓	✓	-
Hypoxylon serpens grey conidial (gc)	✓	✓	✓	✓	-	-
Hypoxylon mammatum	✓	✓	✓	✓	-	-
Hypoxylon multiforme	-	✓	✓	-	-	-
Hypoxylon serpens white silky (ws)	✓	✓	-	-	-	Ridge and Cord-penetration
Rosellinia desmazieresii	✓	✓	-	-	-	-
Rosellinia mammiformis	✓	-	-	-	-	-
"Hypoxylon purpureum"	✓	-	-	-	-	-

Table 4.3. Daldinia concentrica. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:					
		Intermingling	Narrow line	Wide band	Bow-tie	Pincer	Hour-glass
Self	176	100	0	0	0	0	0
1. Intraperithecial	976	1	4	2	13	26	54
Interperithecial							
2. Same stroma	200	0	1	0	15	20	65
3. Different stromata same site	100	0	9	4	59	26	2
4. Different sites	100	0	39	9	28	24	0
Wood/stromatal strains							
5. Same site	86	0	28	35	9	28	0
6. Different sites	88	0	38	11	14	38	0

G tests (Sokal and Rohlf, 1981 a, b) showed that the differences between 2 and 1, 3 and 1, 3 and 2, 4 and 1, 4 and 2, 4 and 3, and 5 and 3 are very highly significant ($p < 0.001$) also between 5 and 6 is significant ($p < 0.05$). There is no significant difference between 4 and 6.

Table 4.4. *Hypoxylon fragiforme*. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:						
		Restricted	Intermingling	Narrow line	Wide band	Bow-tie	Pincer	Hour-glass
Self	139	0	100	0	0	0	0	0
1. *Intraperithecial*	530	20	0.4	17	39	15	5	5
2. *Interperithecial*	100	7	0	33	37	9	12	2
Same stroma								
3. *Wood strains*	419	44	0	45	8	1	0.5	2
Same site								

G tests showed that the differences between 1 and 2, 1 and 3, and 2 and 3 are very highly significant ($p < 0.001$).

Table 4.5. *Hypoxylon nummularium*. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:					
		Intermingling	Narrow line	Wide band	Bow-tie	Pincer	Hour-glass
Self	105	100	0	0	0	0	0
1. *Intraperithecial*	760	1	9	29	32	14	15
Interperithecial							
2. Same stroma	100	0	5	40	36	7	12
3. Different stromata same site	100	0	14	41	18	10	17
4. Different sites	100	0	46	28	5	13	8
Wood strains							
5. Same site	72	0	36	46	10	8	0
6. Different sites	99	0	64	15	9	12	0

G tests showed that the differences between 1 and 2, and 3 and 2 are significant ($p < 0.05$), between 3 and 1, and 6 and 4 are highly significant ($p < 0.01$) and between 4 and 1, 4 and 2, 4 and 3, 5 and 6, and 5 and 3 are very highly significant ($p < 0.001$).

Table 4.6. *Hypoxylon serpens* - grey conidial (gc) mycelial type. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:				
		Intermingling	Narrow line	Wide band	Bow-tie	Pincer
Self	70	100	0	0	0	0
1. *Intraperithecial*	615	0.7	14	35	31	20
2. *Interperithecial*						
Same stroma	100	0	14	52	5	29

G tests showed that the difference between 1 and 2 is very highly significant ($p < 0.001$)

Table 4.7. *Hypoxylon mammatum*. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:				
		Intermingling	Narrow line	Wide band	Bow-tie	Pincer
Self	41	100	0	0	0	0
Intraperithecial	380	0	39	55	2	4
Wood x monospore strain	20	0	0	0	0	100
Same log						

Table 4.8. *Hypoxylon serpens* white silky (ws) mycelial type. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:				
		Intermingling	Narrow line	Wide band	Ridge	Cord-penetration
Self	31	100	0	0	0	0
Intraperithecial	237	0	8	17	45	30

Table 4.9. Rosellinia mammiformis. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing: Intermingling	Narrow line
Self	20	100	0
Intraperithecial	190	0	100 (of which 23% produced conidia along the interaction line)

Table 4.10. *Hypoxylon multiforme* - most strains. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:		
		Intermingling	Wide band	Bow-tie
Self	196	100	0	0
1. *Intraperithecial*	691	100	0	0
2. *Interperithecial*				
Same stroma	11	100	0	0
3. Different stromata same site	125	9	69	22
4. *Wood strains*				
Different logs, same site	13	0	38	62
Wood x monospore strains				
Same log (same decay column)	23	100	0	0
Same log (different decay columns)	23	0	87	13
5. Different sites	682	0	77	23

G tests showed that the differences between 3 and 1, 3 and 2, and 5 and 3 are very highly significant ($p < 0.001$). The difference between 4 and 3 is highly significant ($p < 0.01$) and between 5 and 4 is significant ($p < 0.05$).

Table 4.11. Hypoxylon multiforme - strains from SW1 and perithecia a and c, log AA. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percentage frequency (%) of interactions showing:		
		Intermingling	Wide band	Bow-tie
Self	30	100	0	0
1. Intraperithecial	147	32	46	22
2. Interperithecial				
Same stroma	64	0	73	27
Wood x monospore strains				
3. Same log (same decay column)	2	0	0	100
4. Same log (different decay columns)	17	0	53	47

G tests showed that the difference between 1 and 2 is very highly significant ($p < 0.001$) but between 2 and 4, and 3 and 4 there are no significant differences.

Table 4.12. "Hypoxylon purpureum". Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:	
		Intermingling	Narrow band
Self	48	100	0
Intraperithecial	390	100	0
Wood strains			
Same site	1	0	100
Different sites	20	0	100

Table 4.13. Rosellinia desmazieresii. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing :		
		Intermingling	Narrow line	Wide band
Self	47	100	0	0
Intraperithecial	165	100	0	0
Interperithecial				
Same ring*	3	100	0	0
Different ring*	18	0	11	89
Wood strains				
Different rings*	1	0	100	0
Wood x monospore strains				
Different rings*	14	0	57	43

* R. desmazieresii causes death of Salix repens in annually enlarging ring-like patches of ground (Barrett and Payne, 1982).

Table 4.14. *Hypoxylon fuscum*. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:			
		Intermingling	Trough	Asymmetric lens	Symmetric lens
Self	118	100	0	0	0
1. *Intraperithecial*	120	0	33	2	65
2. *Interperithecial*					
Same stroma	221	0	50	1	49
Wood strains					
3. Same site	245	0	35	7	58
4. Different sites	137	0	57	17	26

G tests showed that the difference between 1 and 2 is significant ($p < 0.05$), and the difference between 4 and 3 is very highly significant ($p < 0.001$).

Table 4.15. *Hypoxylon rubiginosum*. Occurrence of different interaction types in experimental pairings.

Pairing type	Number of interactions tested	Percent frequency (%) of interactions showing:		
		Intermingling	Trough	Raised mycelium
Self	88	100	0	0
1. *Intraperithecial*	660	0.5	94	6
2. *Interperithecial*				
Same stroma	100	0	100	0
Wood strains				
3. Same site	29	0	93	7
4. Different sites	17	0	100	0

G tests showed that the difference between 1 and 2 is highly significant ($p < 0.01$), but there is no significant difference between 3 and 4.

Table 4.16. Values of G for Replicated Goodness of Fit Tests (G statistic) (Sokal and Rohlf, 1981a) for different pairing types in each species.

Comparison between pairing types	Species							
	Daldinia concentrica	Hypoxylon fragiforme	H. nummularium	H. serpens grey conidial (gc)	H. multiforme (most isolates)	H. multiforme (pa, pc and SW1*)	H. fuscum	H. rubiginosum
Intraperithecial with interperithecial, same stroma	26	31	12	41	NA	40	11	12
Interperithecial same stroma, with interperithecial, different stromata	153	NA	12	NA	46	NA	NA	NA
Interperithecial different stromata, same site with interperithecial different stromata, different sites	36	NA	32	NA	NA	NA	NA	NA
Intraperithecial with interperithecial different stromata, same site	168	NA	16	NA	586	NA	NA	NA
Interperithecial same stroma with interperithecial different stromata, different sites	208	NA	89	NA	NA	NA	NA	NA
Intraperithecial with interperithecial different stromata, different sites	211	NA	101	NA	NA	NA	NA	NA
Wood strains same site with wood strains different site	14	NA	21	NA	NA	NA	38	NA

* pa and pc were two perithecia on log AA. This was one of two H. multiforme samples (the other being SW1) which yielded a proportion of somatically incompatible interaction types in intraperithecial and interperithecial pairings. All other H. multiforme samples produced somatically compatible ascospore progeny (see text).

Table 4.17. Daldinia concentrica. Genetic designation of hyphal tips from interaction zones.

Interaction type	Genotypes paired*	Source of hyphal tip †	Genotypes recovered after (d)*				
			7	14	21	28	35
Hour-glass	3,1	I	1	3	1	3	1
		O	3	3	3	3	3
	3,2	I	3	2	3	3	2
		O	3	B	3	3	3
	4,2	I	4	B	2	2	2
		O	B	2	4	B	B
	5,1	I	1	5	B	5	B
		O	B	5	B	1	1
Bow-tie	2,1	I	1	2	1	2	B
		O	2	1	2	1	2
	4,1	I	1	1	1	1	1
		O	4	1	4	1	1
	5,2	I	5	5	5	5	2
		O	2	2	5	5	2
Pincer	4,3	I	3	4	4	3	B
		O	3	3	3	3	3
	5,3	I	3	3	5	3	3
		O	3	5	5	3	3
	5,4	I	4	B	4	5	5
		O	4	4	B	4	4

* Genotypes are assigned by strain number. B indicates that both were recovered.

† Hyphal tips were obtained either from I (between inoculum plugs) or O (2 cm away).

Table 4.18. Hypoxylon fragiforme. Genetic designation of hyphal tips from interaction zones.

Interaction type	Genotypes paired*	Source of hyphal tip†	Genotypes recovered after (d)*				
			7	14	21	28	35
Wide band	9,4	I	B	B	4	B	9
		0	B	9	4	4	9
	9,8	I	8	9	9	8	B
		0	8	9	9	8	8
Hour-glass	7,4	I	7	4	7	4	4
		0	7	4	4	7	4
	9,5	I	5	5	5	5	9
		0	5	B	B	5	5
	10,4	I	10	10	B	4	4
		0	4	4	B	10	B
Bow-tie	7,5	I	5	7	7	B	B
		0	5	7	7	B	7
	8,2	I	8	2	8	2	2
		0	8	2	2	8	B
	9,2	I	9	9	9	9	2
		0	2	2	2	9	2
	10,3	I	3	10	3	10	3
		0	10	10	3	3	3
Pincer	7,2	I	2	2	7	7	7
		0	2	7	7	7	7

* Genotypes are assigned by strain number. B indicates that both were recovered.
† Hyphal tips were obtained either from I (between inoculum plugs) or 0 (2 cm away).

Table 4.19. Hypoxylon nummularium. Genetic designation of hyphal tips from interaction zones.

Interaction type	Genotypes paired*	Source of hyphal tip†	Genotypes recovered after (d)*				
			7	14	21	28	35
Narrow line	4,3	0	3	4	4	3	3
	7,11	0	B	7	11	7	7
Wide band	5,2	0	5	5	2	5	5
	5,3	0	5	5	3	3	3
	5,15	0	15	5	15	B	15
	6,14	0	14	6	14	14	6
	17,11	0	B	17	17	17	11
	19,20	0	19	19	20	20	20
Bow-tie	2,1	0	1	1	2	B	1
	3,1	0	1	1	3	3	3
	3,2	0	2	3	B	3	3
	4,1	0	B	4	4	B	4
	4,2	0	2	4	4	4	2
	5,1	0	B	1	5	5	B
	5,4	0	5	4	4	5	4
	10,20	0	10	10	20	20	10
Pincer	8,14	0	14	14	14	14	14
	9,20	0	9	9	20	20	9
	16,14	0	16	14	14	14	14
	17,20	0	17	20	17	17	17

* Genotypes are assigned by strain number. B indicates that both were recovered.

† Hyphal tips were obtained from 0 (2 cm away from inoculum plugs).

Table 4.20. Hypoxylon serpens (white silky - ws - mycelial type). Genetic designation of hyphal tips from interaction zones.

Interaction type	Genotypes paired*	Source of hyphal tip†	Genotypes recovered after (d)*				
			7	14	21	28	35
Wide band	8,3	I	3	8	8	3	B
		O	3	3	8	3	B
	15,12	I	12	15	15	B	B
		O	12	15	15	15	B
Ridge	6,4	I	6	6	6	6	4
		O	6	4	6	4	6
	10,9	I	10	9	9	B	9
		O	9	9	9	9	B
	18,6	I	B	6	B	18	B
		O	B	6	6	18	18
	19,17	I	19	17	17	19	19
		O	19	19	17	19	19
Cord-penetration	7,4	I	7	7	7	7	7
		O	7	7	7	7	7
	14,6	I	6	6	6	6	6
		O	6	6	6	6	6
	19,7	I	19	19	19	19	19
		O	19	19	19	19	19
	19,12	I	12	19	19	19	19
		O	12	19	19	B	19

* Genotypes are assigned by strain number. B indicates that both were recovered.
† Hyphal tips were obtained either from I (between inoculum plugs) or O (2 cm away).

Table 4.21. Hypoxylon multiforme. Genetic designation of hyphal tips from interaction zones.

Interaction type	Genotypes paired*	Source of hyphal tip†	Genotypes recovered after (d)* 43
Bow-tie	2,R	0	R
	2,4	0	2
	2,5	0	2
	2,F5	0	F5
	4,R	0	R
	4,S	0	4

* Genotypes are assigned by strain number.

† Hyphal tips were obtained from 0 (2 cm away from inoculum plugs).

Table 4.22. Hypoxylon fuscum. Genetic designation of hyphal tips from interaction zones.

Interaction type	Genotypes paired*	Source of hyphal tip †	Genotypes recovered after (d)* 166
Trough	3,1	I	3
		O	3
	4,1	I	4
		O	4
	9,6	I	9
		O	9
Asymmetric lens	8,2	I	2
		O	2
Symmetric lens	4,2	I	B
		O	B
	4,3	I	4
		O	4
	6,2	I	6
		O	6
	8,1	I	B
		O	B
	9,3	I	9
		O	9
	9,8	I	9
		O	9

* Genotypes are assigned by strain number. B indicates that both were recovered.

† Hyphal tips were obtained either from I (between inoculum plugs) or O (2 cm away).

Table 4.23. Genetic designation of single conidia from interaction zones.

Species	Interaction type	Genotypes paired*		Number of single conidial colonies (10 total) of genotype	
		A	B	A	B
Hypoxylon mammatum	Narrow line	$^{1}_{10}$	$^{1}_{3}$	4	6
		$^{2}_{17}$	$^{2}_{3}$	0	10
	Wide band	$^{1}_{13}$	$^{1}_{6}$	6	4
		$^{1}_{15}$	$^{1}_{4}$	5	5
		$^{1}_{12}$	$^{1}_{2}$	6	4
		$^{2}_{7}$	$^{2}_{1}$	2	8
		$^{2}_{10}$	$^{2}_{5}$	1	9
		$^{2}_{10}$	$^{2}_{6}$	7	3
	Bow-tie	$^{2}_{17}$	$^{2}_{6}$	2	8
	Pincer	$^{2}_{15}$	$^{2}_{9}$	10	0
Rosellinia mammiformis	Narrow line	9	6	5	5
		8	7	8	2
		10	7	8	2
		5	19	7	3
		17	15	10	0

* Genotypes are assigned by strain number.

Table 4.24. Phenol oxidase and peroxidase activity of the interaction zones between some xylariaceous species recorded after 6 h and 30 min respectively.

Species	Interacting strains (mycelial type)	Phenol oxidase						Peroxidase
		Salicylic acid	Quinol	Catechol	Guaiacol	Ferulic acid	Dihydroxy-phenylalanine	
Daldinia concentrica	as1 x w1	NR	NR	NR	NR	NR	NR	++
Hypoxylon fragiforme	as1 x as2	NR	NR	NR	C (coral)	NR	C (dark brick-faint)	+++
	w1 x as2	NR	NR	NR	C (coral)	NR	C (coral)	++
	w1 x w2	NR	NR	NR	NR	NR	C (coral)	++
H. mammatum	as1 x as2	NR	C (cinnamon)	C (dark mouse grey)	C (brick	NR	C (isabelline)	+++
H. multiforme	as1 x as2	NR	C (rosy buff)	NR	NR	NR	NR	+
H. nummularium	as1 x as2	NR	C (coral-faint)	NR	NR	NR	NR	+++
	as3 x as4	NR	NR	NR	NR	NR	NR	+++

Origin of strains

as ascospore
w wood
c conidial

Mycelial types

ws white silky
gc grey conidial

Reactions

NR no reaction
C colour recorded in agar around wells, Colour recorded in brackets
\+ gas production around well periphery
++ gas production over whole surface of well
+++ gas production up to 1 mm above surface of well

Table 4.24. (continued).

Species	Interacting strains (mycelial type)	Phenol oxidase					Peroxidase
		Salicylic acid	Quinol	Catechol	Guaiacol	Ferulic acid	Dihydroxy-phenylalanine	
"H.purpureum"	as1 x c1	NR	C (coral-faint)	NR	NR	NR	NR	+++
	as3 x as4	NR	C (coral-faint)	NR	NR	NR	NR	+++
H. rubiginosum	as1 x w1	NR	C (rosy vinaceous)	NR	NR	NR	NR	+
	as2 x as3	NR	C (rose)	NR	NR	NR	NR	+
H. serpens	as1 x as2	NR	NR	NR	NR	NR	NR	++
	as6 x as7 (ws)	NR	NR	NR	NR	NR	NR	+++
	as8 x as9 (gc)	NR	NR	NR	NR	NR	NR	++

Origin of strains

as ascospore
w wood
c conidial

Mycelial types

ws white silky
gc grey conidial

Reactions

NR no reaction
C colour recorded in agar around wells. Colour recorded in brackets
+ gas production around well periphery
++ gas production over whole surface of well
+++ gas production up to 1 mm above surface of well

Figure 4.1. Aerial mycelium interactions between strains of Daldinia concentrica.

(A,B)"Intermingling"(A viewed from above, B viewed from below). The original zone of contact between the two colonies is indiscernible. (C,D)"Narrow line"(C from above, D from below). A narrow ($\leqslant$ 3 mm) line of woolly-white aerial mycelium (am) is discernible between the colonies, underlain by an amber pigmented zone (**p**). (E,F)"Wide band"(E from above, F from below). A wide (> 3 mm) zone of more or less constant width containing an am is bounded by fuscous black pigmented regions (**p**).

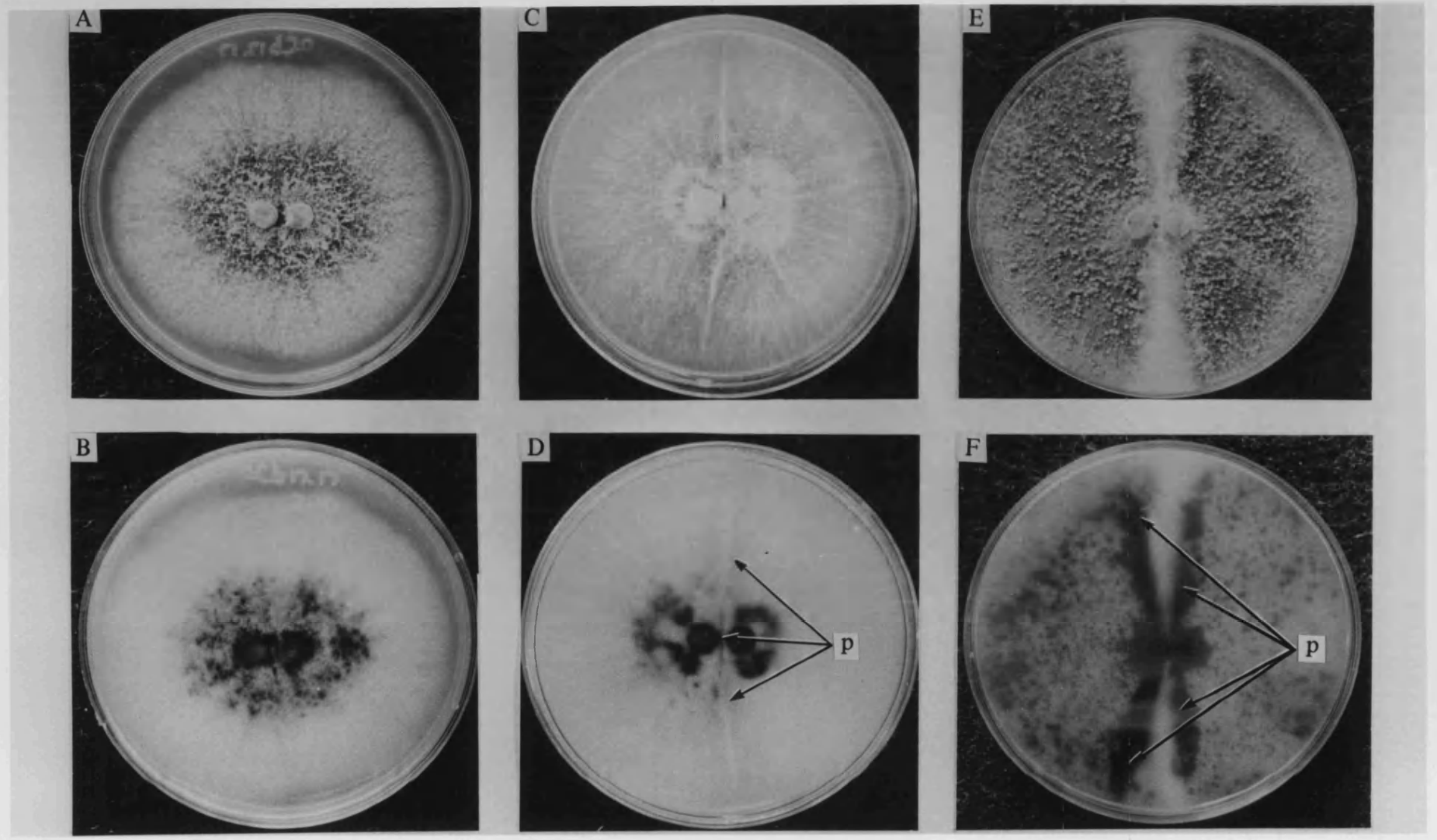

A
C
E
B
D
p
F
p

Figure 4.2. Aerial mycelium interactions between strains of Daldinia concentrica. (A-C) "Bow-tie". Early in development (A) the parent colony margins are inhibited allowing emergence of a fan-shaped aerial mycelium (am). In (B) and (C) (viewed from above and below respectively) a symmetric bow-tie has been produced as a result of restriction of the parent colony margins to an equal extent. (D-F) "Pincer". Early in development (D) extension of only one of the parent colony margin is suppressed, resulting in unilateral outgrowth of the am. In (E) and (F) (viewed from above and below respectively) the fully developed pincer shows the non-suppressed isolate (**n**) has gained territorial dominance, partially encircling the suppressed colony (**s**). (G,H) "Hour-glass". (G from above, H from below). A symmetric am, constricted at both its centre and periphery, and delimited by fuscous black pigmented regions (**p**) is visible between the colonies. The strains paired in (A) and in (D) are different from those paired in (B) and (C), and (E) and (F) respectively.

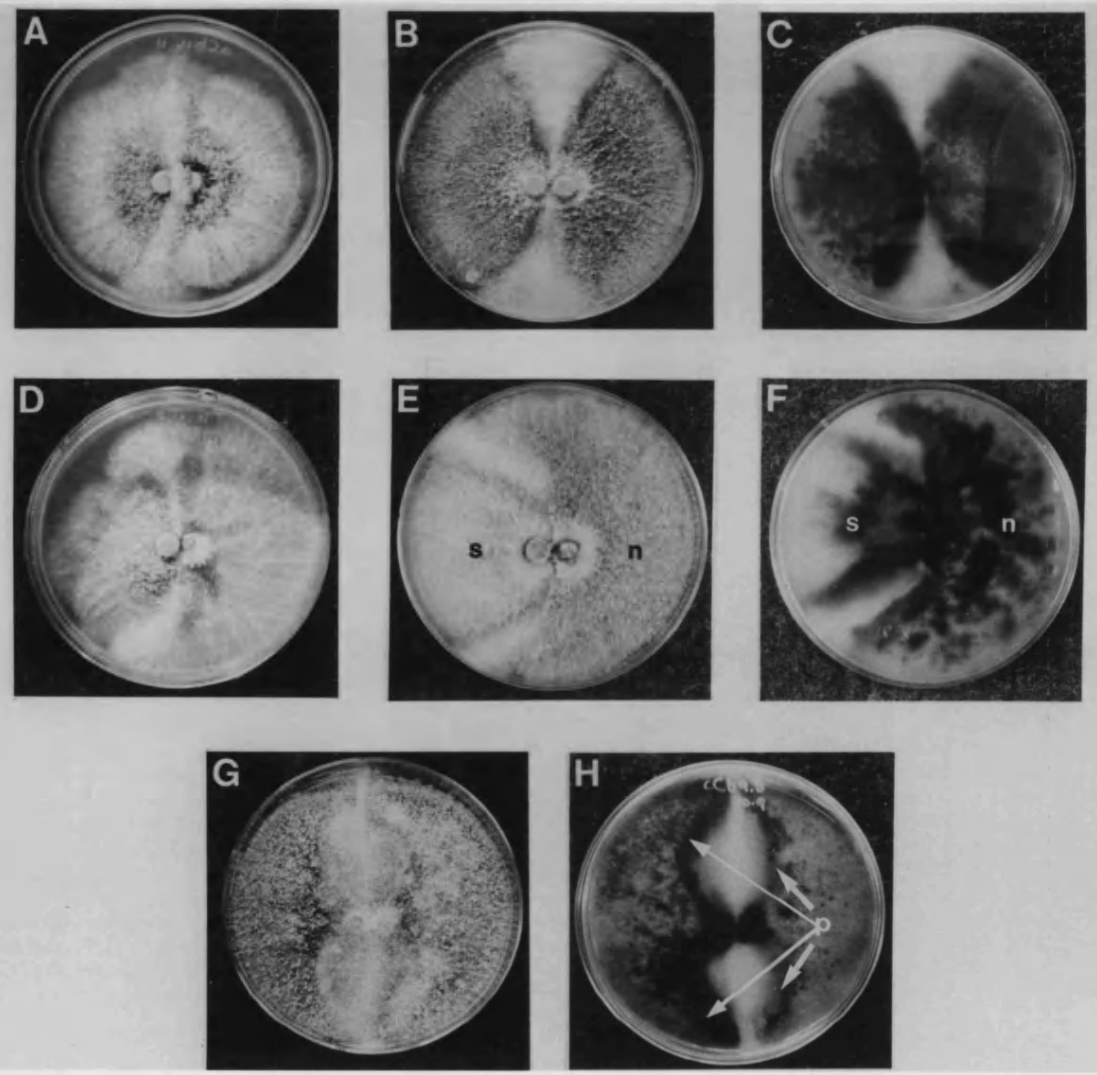

A
B
C
D
E
s
n
F
s
n
G
H

Figure 4.3. Aerial mycelium interactions between strains of Hypoxylon serpens (white silky - ws - mycelial type). (A,B) "Ridge" (A viewed from above, B viewed from below). A ridge, at least 3 mm high and 5 mm wide of white aerial mycelium (am) separates the two colonies and is bounded underneath by pseudosclerotial plates (PSPs). Note how the ridge has spread into the colony which has not produced a well-defined PSP. (C,D) "Cord-penetration" (C viewed from above, D viewed from below). White hyphal aggregations or cords extend from the interaction zone into one colony and not the other which has produced a PSP along the line of contact.

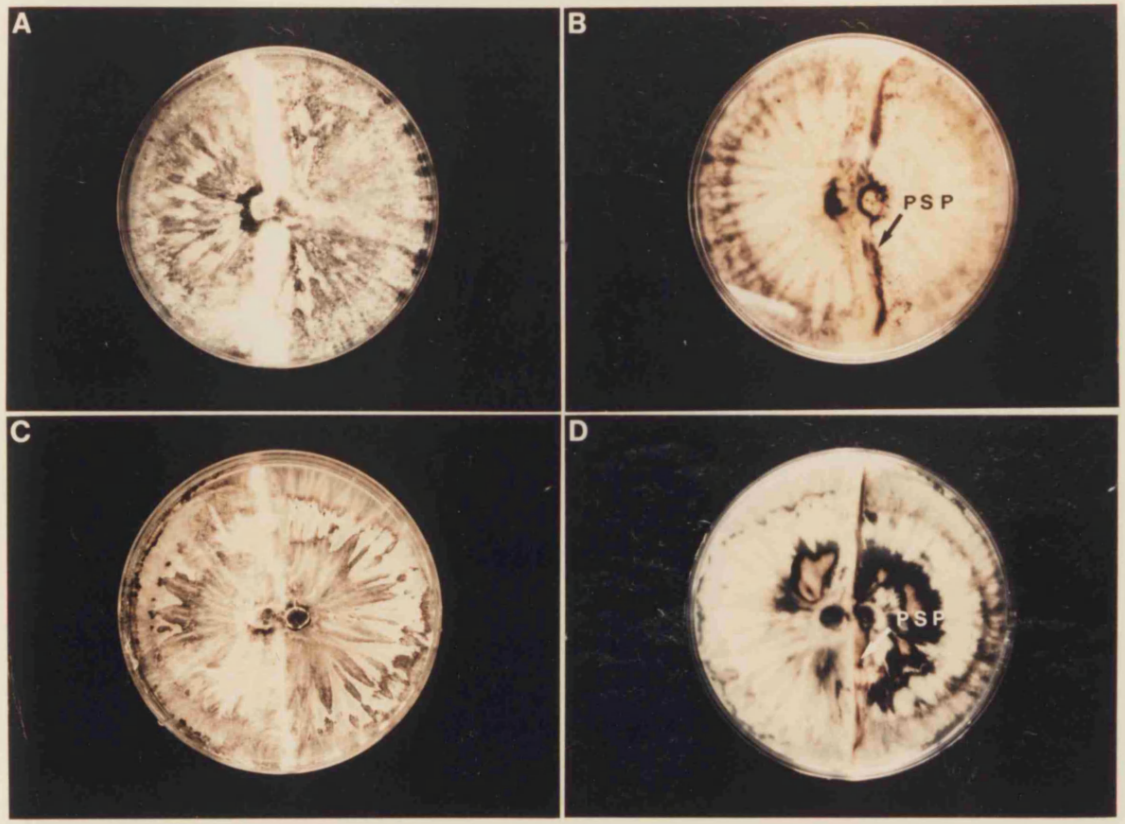

A
B
PSP
C
D
PSP

Figure 4.4. Trough or barrage interactions between strains of Hypoxylon fuscum (A,C) and Hypoxylon rubiginosum (B,D). "Trough" (A,B). A trough (< 2 mm) of sparse mycelium pigmented isabelline in H. fuscum (A) and sepia in H. rubiginosum (B) separates the colonies. "Lens" (C). An oval or lens-shaped area of powdery buff to fawn mycelium exists between the H. fuscum colonies which developed initially from a buff mycelium produced in the trough, became vinaceous buff to fawn and widened asymmetrically. "Raised mycelium" (D). A pale luteus powdery mycelium raised by at least 1 mm above the adjacent colonies separates the H. rubiginosum colonies. This developed as a white mycelium in the hollow of the trough zone and enlarged to become a ridge.

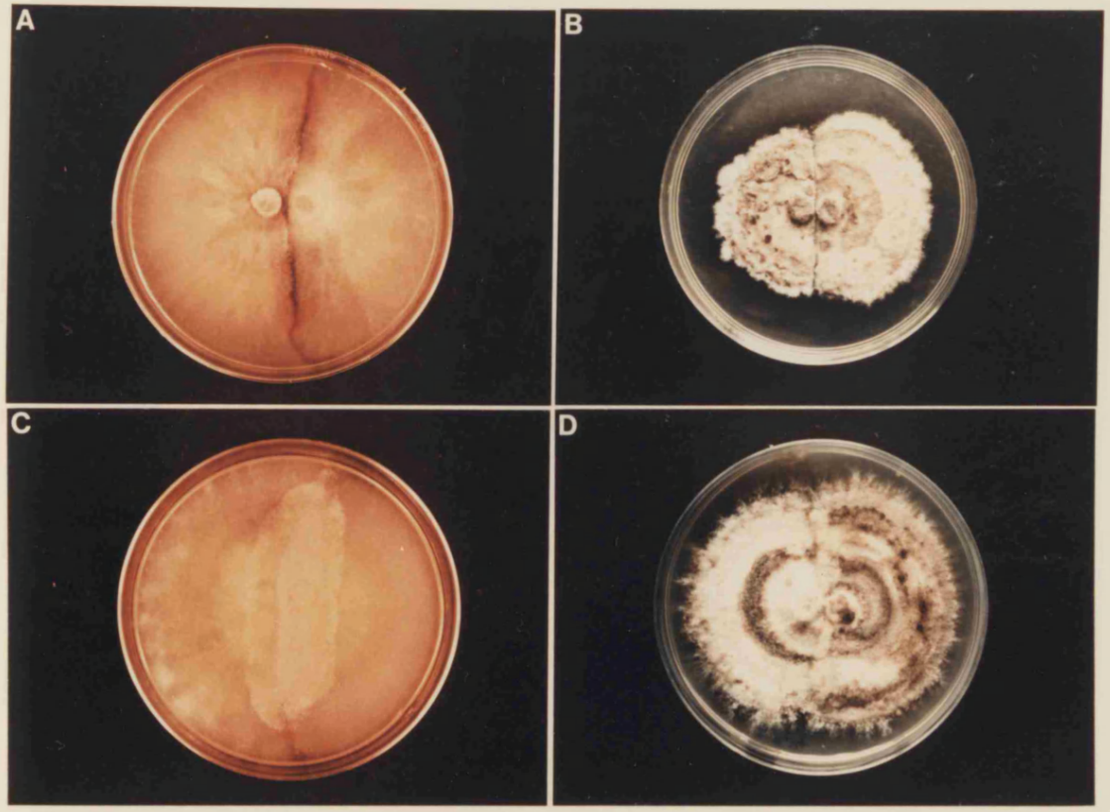

A
B
C
D

Figure 4.5. Genetic designation of hyphal tips from interaction zones as demonstrated in Hypoxylon nummularium. (A). The colony arising from the hyphal tip (in the centre of the Petri dish) obtained from the interaction zone has grown out uniformly and intermingled with one (**b**) of the two types originally paired, whilst producing a reaction zone against the other (**a**). The hyphal tip colony can therefore be assigned to strain **b**. (B) The hyphal tip colony has grown out uniformly but interacted against both original strains. (C) The hyphal tip has sectored into the two original types (as shown by backcrosses between subcultures from each sector and the original strains).

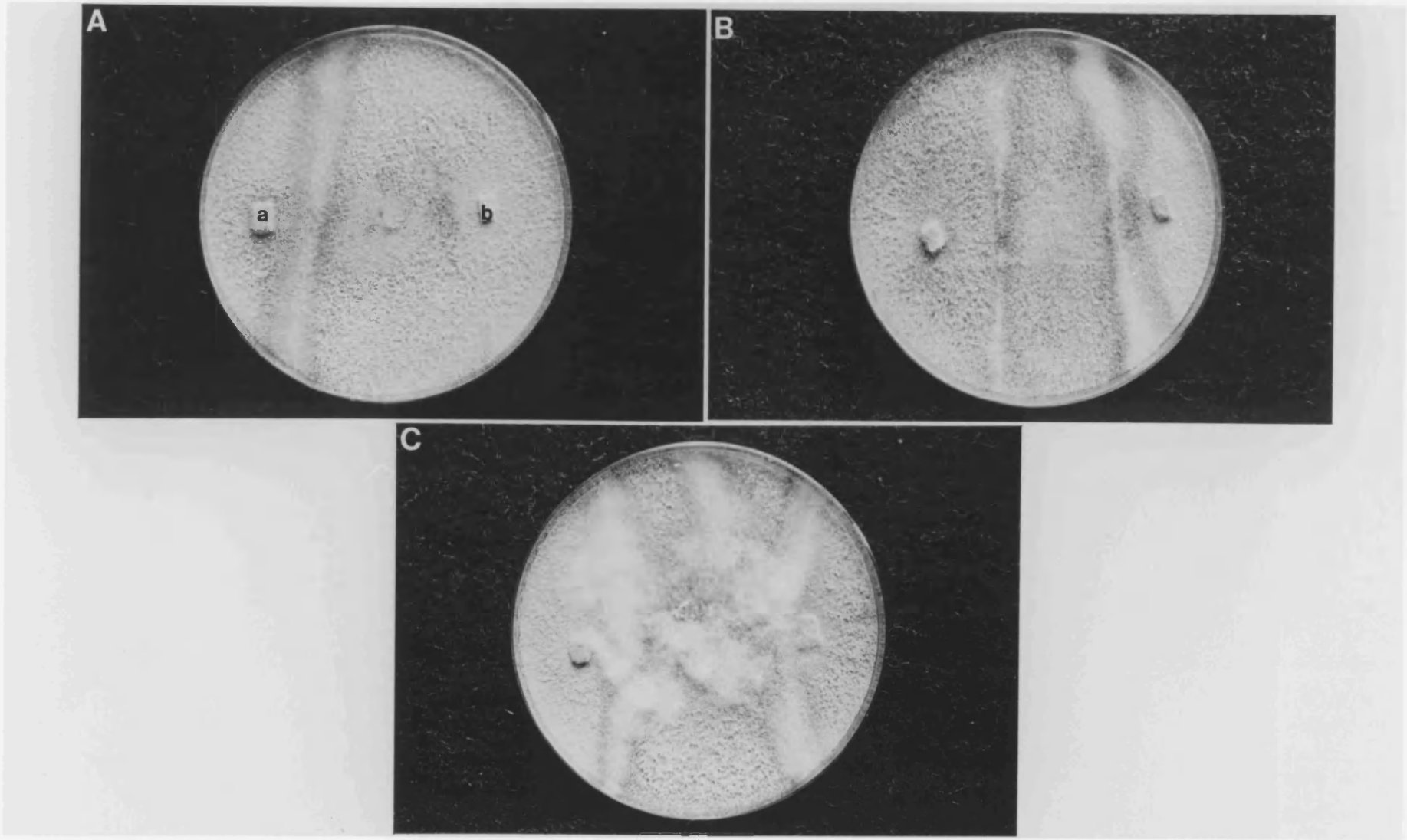
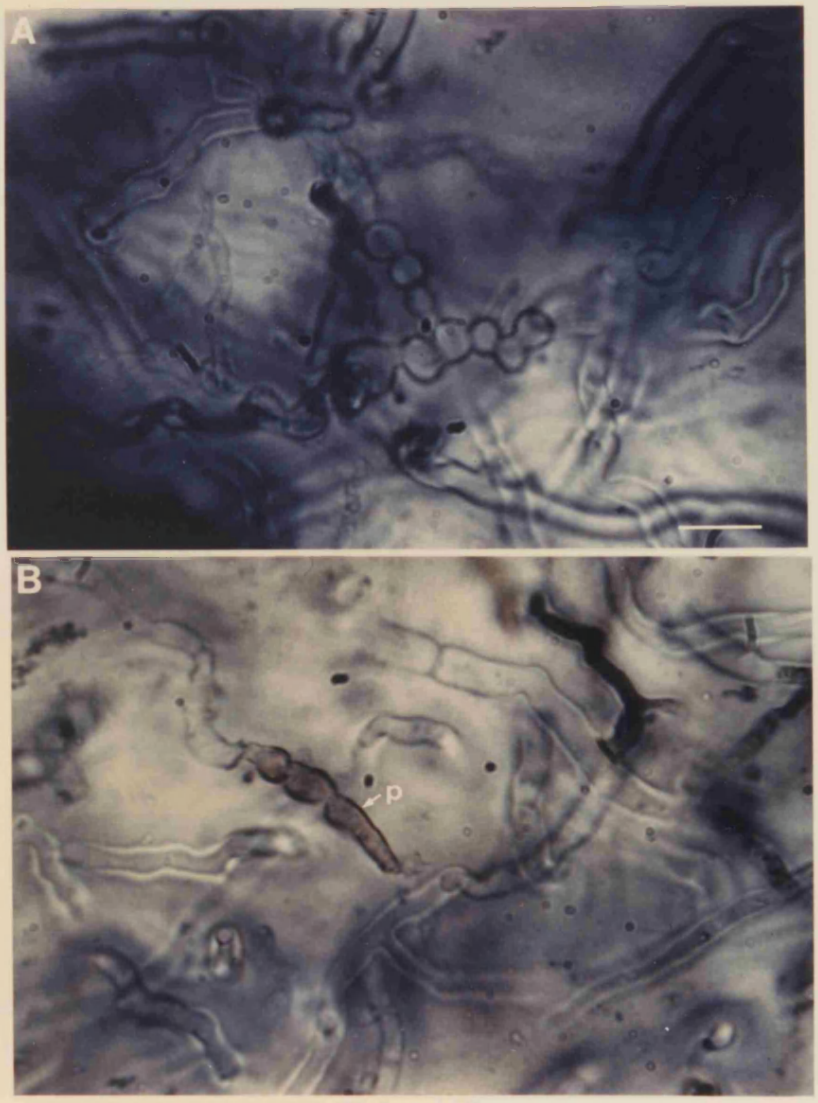

A
a
b
B
C

Figure 4.6. Tightly packed hyphae with spherical or tubular swellings in a pseudosclerotial plate of Hypoxylon mammatum. Note the pigmentation (**p**) of the swollen hypha in (B). Bar marker represents 10 µm.


A
B
p

CHAPTER 5

POPULATION STRUCTURE

5.1 Introduction

Analysis of the distribution and variation of genotypes (identified on the basis of somatic incompatibility - see Chapter 4) in the field provides an insight into the spatial and genetic structure of fungal populations in nature. Further, such investigations can lead to an understanding of possible patterns of dispersal and establishment, including invasion and colonization strategies (Rayner and Todd, 1982; Rayner and Boddy, 1986).

The structure of a population will depend on the pattern of breeding biology adopted. Sexual outcrossing results in variable populations, whilst sexual non-outcrossing and asexual reproduction produce clonal populations composed of genetically identical or very similar individuals (Rayner and Todd, 1982; Rayner and Boddy, 1986). There is evidence that populations of some heteromictic (heterothallic) ascomycetes have both outcrossing and clonally-derived structures. This has been found for Neurospora crassa (Yassin, 1980), Cryptostroma corticale (Bevercombe, 1980), Ophiostoma ulmi (Brasier, 1984) and Diaporthe phaseolorum (Ploetz and Shokes, 1986). In these populations a few genotypes are widespread, but unique genotypes with a limited distribution, also exist.

This chapter reports on the spatial and genetic population

structure of a number of xylariaceous species and discusses the possible patterns of breeding biology, dispersal and establishment they may adopt.

An attempt to induce Hypoxylon mammatum, Rosellinia desmazieresii and Daldinia concentrica to produce perithecia in culture is also described. A knowledge of their mating systems (i.e. whether they were homomictic or heteromictic and in the latter which strains were compatible with which) would confirm what is otherwise informed speculation (arising from the results of intraspecific interactions between ascospore strains isolated from the same perithecium, discussed in Chapter 4, Section 4.4). Further, if viable ascospores of D. concentrica could be produced, then a backcross experiment involving the elimination of somatic incompatibility factors (see Chapter 1, Section 1.1, ii,(b)) could be conducted, as was performed in Ophiostoma ulmi (Brasier, 1984). This may provide three pieces of evidence. Firstly whether somatic incompatibility is determined by a polygenic mechanism (suggested in Chapter 4, Section 4.4). Secondly whether the somatic incompatibility loci are functionally independent of the mating type locus. Thirdly, whether there is a relationship between interaction phenotype and genetic relatedness (i.e. that somatic rejection increases and conversely acceptance (access) decreases with increasing genetic differences between isolates- a proposal made in Chapter 4, Section 4.4).

5.2 Materials and Methods

i. Analysis of genotype distribution in nature

Portions of decaying wood bearing perithecial stromata of various xylariaceous species (*Daldinia concentrica*, *Hypoxylon fragiforme*, *H. fuscum*, *H. multiforme*, *H. nummularium*, "*H. purpureum*", *H. rubiginosum*, *H. serpens* and *Rosellinia desmazieresii*) were collected from the sites listed in Chapter 2, Table 2.1. The samples were sectioned transversely with a band saw and the position of stromata and distribution of decay were recorded diagrammatically or photographically. The fungi were isolated into pure culture (using the procedures described in Chapter 2, Section 2.3, i, ii and iii) from 10 mm^3 wood fragments from the centre of decay zones, and also from single ascospores, and in *D. concentrica* from stromata.

The wood sections were stored separately at room temperature for up to 14 d with 5 ml sterile distilled water in sealed polythene bags, in order to encourage mycelial growth so that the distribution of mycelia could be recorded.

Experimental pairings were made between various combinations of strains using the methods described in Chapter 2, Section 2.5.

ii. Perithecial production in culture

Ten single ascospore strains isolated from the same perithecium of *Hypoxylon mammatum* were experimentally paired (as described in Chapter 2) on cornmeal agar (CMA; 17 g corn meal agar (Oxoid code

no. CM103) and 1000 ml distilled water, autoclaved at 121° C for 15 min) in 9 cm Petri dishes, a procedure successfully used to produce perithecia in Nectria species (Ian Parrett, pers. comm.). Five single ascospore strains of R. desmazieresii were also inoculated individually onto CMA. Both species were incubated at 20°C in darkness. Hypoxylon mammatum and R. desmazieresii were selected for this experiment as they have relatively little stromatic tissue and in Nectria, species with this feature produce perithecia more abundantly than those with larger stromata.

For D. concentrica a different method was used. Conical flasks (250 ml) were filled with 4 g of wood shavings (ashwood drilled from a freshly felled healthy tree) or paper*(torn into 20 cm^2 pieces) and 100 ml of a medium containing 0.2% (w/v) malt extract and 0.8% (w/v) agar (i.e. 2 g malt extract and 8 g agar in 1000 ml distilled water). Flasks were plugged with cotton wool and muslin bungs and autoclaved at 121°C for 45 min (wood) or 30 min (paper).

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* Ten types of paper were tested for lignin which is detected by a cherry-red colour (Weisner reaction) with phloroglucinol solution (4 g phloroglucinol - BDH Chemicals Ltd., Poole, Dorset -in 100 ml absolute ethanol and 50 ml concentrated hydrochloric acid) (Walker, 1975). Most papers did not react at all and of those that did the most intense staining was obtained with newspaper-quality paper.

Ten single ascospore strains derived from the same perithecium were checked for conidial production in culture and were then paired in all combinations in the flasks prepared above. One strain of each pair was inoculated as a 6 mm diameter plug of agar plus mycelium and allowed to grow for 7 d before the other strain was inoculated as a conidial suspension (0.1 ml). This was obtained by agitating (Fisons Whirlimixer) 1 cm^3 agar plus conidia-bearing mycelium in 2 ml sterile distilled water. Preliminary studies in which the density of the conidial suspension was altered, showed that this ratio of mycelium to water allowed optimal germination. Following the addition of the suspension, each flask was gently swirled to ensure an even distribution of conidia over the medium/colony surface. Control flasks, inoculated with the conidial suspension only, were prepared for each strain in addition to self pairing controls. All flasks were incubated in the laboratory (away from direct sunlight) at room temperature for 7 months. During this period (after 3 months) they were treated for 3 d at 4°C in darkness.

5.3 Results

i. Breeding biology

As described in Chapter 4 (Section 4.4), intraperithecial pairings in all species except for H. multiforme, "H. purpureum" and R. desmazieresii resulted in a high proportion of somatically incompatible reactions. That is single ascospore strains derived from the same perithecium were genetically different with respect to their somatic incompatibility loci. This indicates that these

species are outcrossing (i.e. fertilization occurs between genetically different individuals). Single ascospore strains derived from the same perithecium in H. multiforme (in all but two samples), "H. purpureum" and R. desmazieresii however, were all somatically compatible. That is they were genetically identical with respect to their somatic incompatibility loci, suggesting that these species are non-outcrossing (i.e. the homokaryon is self-fertile). As the distribution of genotypes in nature is a direct consequence of outcrossing or non-outcrossing, species with these alternative breeding strategies will be considered separately.

(a) Outcrossing species

Genotype distribution within a single resource unit

The distribution of individual genotypes was ascertained by combining evidence from the presence of interactive zone lines in wood and the occurrence of somatic incompatibility between strains in culture.

Strains were somatically compatible when they were isolated from different parts of the same decay column (except H. fragiforme and H. fuscum - see below). They were somatically incompatible (producing a variety of interaction types - see Chapter 4, Section 4.3, i, (a) and (b)) when they were isolated from different decay columns. Occasionally strains from different decay columns intermingled (for example D. concentrica logs II and VI). Where this occurred the line separating the two columns appeared grey and diffuse by comparison with the distinct black lines that usually

bounded different zones. As species other than the xylariaceous ones were always isolated from adjacent zones, the diffuse lines were considered to be relics of past confrontations by which the xylariaceous species had gained territory from the other fungus.

Daldinia concentrica strains were isolated from 13 logs collected from three sites (Bathwick Woods, Friary Woods and Ashclyst Forest). In 12 of these a limited number of genotypes, often only one, appeared to occupy the entire length and cross-sectional area (Table 5.1). However, log VII, from which 30 genotypes were isolated, was an exception. This was a 12 m long, dead, wind-thrown trunk whose roots were partially decayed by Armillaria species. A detailed study (Figure 5.1) revealed that for much of the length of the log a few genotypes predominated. Some occupied more than 50% of the cross-sectional area of the trunk and extended up to 4.95 m (genotype 4) and 3.24 m (genotype 9). Other genotypes appeared to occupy comparatively small domains, for example the large number of small individual mycelia present in sections KL and NO. Interactive zone lines between these genotypes were not evident in the wood.

Strains isolated from D. concentrica stromata were somatically compatible with those isolated from decay columns in wood corresponding to the position of the stroma. However, they were somatically incompatible with strains isolated from other decay columns. Generally those genotypes that occupied large volumes of wood produced the most stromata, for example a genotype extending for 2.93 m in log VII produced six stromata (Table 5.1).

The distribution of H. rubiginosum genotypes within ash logs from two sites (Friary Woods and Ashclyst Forest) was similar to that of D. concentrica, in that only one or two types existed per log (Table 5.2). Usually only one genotype was isolated from each cross-section and the maximum genotype length recorded was 1 m. Of the seven logs studied, six were colonized by H. rubiginosum alone, but a single genotype of D. concentrica was also present in log RF. This occupied the whole of an end-section, whilst the two H. rubiginosum genotypes each appeared to colonize other whole cross-sections and extended for 0.6 m and 0.25 m (Figure 5.2).

Whilst resource units colonized by D. concentrica and H. rubiginosum contained, with one exception (log VII), few genotypes, each occupying a considerable volume, those colonized by H. fragiforme and H. fuscum supported several genotypes in relatively small domains (Tables 5.3 and 5.4). The number of H. fragiforme genotypes in each of four logs from three sites (Ashclyst Forest, Venbridge and Colerne Woods) varied from seven to 51 with at least three to 13 in each log section (compared to usually one or two in D. concentrica or H. rubiginosum). This is probably a fraction of the true numbers, as these figures were the result of single isolations from each decay zone, and many of these yielded mycelia that sectored into different genotypes. Only once (log AS1) was a particular genotype found in more than one log section. In addition, although for example in log VW the maximum genotype length was estimated as less than 4 m, this may well be an over-estimate as it was based on the distance (2 m) between sample cross-sections

(Figure 5.3). Sometimes, as in D. concentrica, interactive zone lines were not apparent between different genotypes. For example in log VW, section B, genotypes 9 and 10 were isolated from more than one position, often with other genotypes too, indicating that they may be so irretrievably intermixed that they are unable to form discrete decay columns.

Similarly, in H. fuscum nine genotypes were identified for a decay area in one cross-section of pole 15. In this species 14 logs were sampled from five sites (Friary, Venbridge and Manor Woods, Sutton Farm and Castle Combe) and the number of genotypes in a log varied from one to 16 which spread lengthwise for 0.3 to 1.7 m with as many as nine genotypes occupying a single cross- section of wood (Table 5.4).

A maximum of nine genotypes per log were isolated from beech (Fagus sylvatica) wood colonized by H. nummularium (from four sites - Clifford Bridge, Venbridge, Colerne and Friary Woods). Three genotypes (each from a different decay zone) were found in one cross-section, although some genotypes extended for considerable lengths, for example up to 1.3 m (Table 5.5).

Wood isolates of H. serpens appeared to be difficult to obtain as a variety of faster fungi, notably Trichoderma species, swamped isolation plates of four logs bearing H. serpens stromata. This species was however successfully isolated from two small (0.3 m and 0.5 m long) wood samples and both were occupied by a single genotype.

Four logs of different tree species (log RF - Fraxinus excelsior, logs F4 and F5 both Corylus avellana and log VB - Betula sp.) from two sites (Friary and Venbridge Woods) yielded not only isolates of the Hypoxylon species that had produced stromata, but also isolates that were typical of H. nummularium (Table 5.5; N.B. logs S and HM2 in this table are discussed later with Hypoxylon multiforme - Section 5.3, i, (b)). These isolates were experimentally paired together and were assigned genotypes which were usually found only in particular decay zones of one cross-section of a log, indicating that their domains were small. Schematic diagrams of these logs show the position of these genotypes in relation to those of other species (Figures 5.2, log RF; 5.4, log F4; 5.5, log VB; 5.8, log F5).

Genotype distribution between resource units

Each particular genotype of D. concentrica, H. rubiginosum, H. fragiforme, H. fuscum and H. nummularium was found to be restricted to a single resource unit (log) within a site. Pairings between strains derived from different logs within and between sites always resulted in somatically incompatible interaction types (see Chapter 4, Tables 4.3, 4.15, 4.4, 4.14 and 4.5, respectively).

(b) Non-outcrossing species

Since the population structures of the non-outcrossing species (H. multiforme, "H. purpureum" and R. desmazieresii) differed from one another in certain characteristic details, each will be considered individually.

Hypoxylon multiforme

Intraperithecial pairings between most H. multiforme strains (26 samples from 13 sites) resulted in the intermingling (somatically compatible) interaction, indicating that this species is non-outcrossing. However intraperithecial pairings between strains from a stroma collected from Sweden, and from two perithecia (pa and pc) on log AA from Ashclyst Forest produced some somatically incompatible reactions. This suggests that occasionally outcrossing takes place. A proportion of the single ascospore strains isolated from each of these perithecia did intermingle when paired, so that these isolates appeared to share the same genotype (with respect to somatic incompatibility loci). For example the 14 Swedish ascospore strains fell into six genotypes (nine strains were of a single genotype) and the eight strains from each of pa and pc could be assigned to five and four genotypes respectively (a maximum of three strains of the same genotype in each case).

With respect to the majority of samples, that is non-outcrossing H. multiforme, a single genotype was present in each of six out of seven logs bearing stromata of this species (Table 5.6). Two of these genotypes occupied large volumes of wood - 2 m and 2.4 m in length. When single ascospore strains were isolated from the same log as wood strains, they intermingled (were somatically compatible) when paired. In the seventh log (HM2) it appeared that two genotypes occupied the wood, as when paired together they consistently failed to intermingle and instead produced a wide band (somatically incompatible) interaction. This log was one of three (HM2, S and F5) bearing H. multiforme stromata, from two sites

(Friary Wood and Clifford Bridge), from which not only H. multiforme was isolated, but also mycelia typical of H. nummularium and/or "H. purpureum"; a situation described for some other Hypoxylon species (see Section 5.3, i, (a)). As in those logs, these mycelia probably occupied only small volumes of wood, as they were usually isolated from a decay zone in a single cross-section. Figures 5.6, 5.7 and 5.8 show the position of these mycelia in relation to those of H. multiforme (log S), H. multiforme and Stereum hirsutum (log HM2) and H. multiforme, H. fuscum and Hymenochaete corrugata (log F5) respectively.

A detailed investigation into genotype distribution in log AA, one of the outcrossing H. multiforme samples, revealed an intricate relationship between the position of three genotypes in a small volume of wood (30 cm long, 4.5 cm diameter) (Table 5.6) and the perithecia they produced (Figure 5.9). Intraperithecial pairings of single ascospore strains isolated from eight perithecia (pa-ph) showed that only progeny from pa and pc, adjacent perithecia on the same stroma, were the product of outcrossing. Progeny from each of the other perithecia, including ph on the same stroma as pa and pc, were non-outcrossing (i.e. "typical" of H. multiforme). Single ascospore strains isolated from these "non-outcrossing" perithecia intermingled with the genotype isolated from wood corresponding with the position of each perithecium, whilst producing a reaction zone (wide band or bow tie) with genotypes in the wood elsewhere. Hence pd progeny were somatically compatible with genotype 2, pb, pf, pg and ph with 1, and pe with 3. Progeny from pa were however somatically incompatible with all three wood genotypes. They also

produced incompatible reactions with each other, and in all interperithecial pairings. This was also the situation for most (five out of eight) of the progeny of pc. The remaining progeny (three out of eight) were somatically compatible with one another, with wood genotype 2 and with pd ascospore strains, although they were incompatible with all other strains.

Other samples collected from the same sites as those supporting the "outcrossing" perithecia, were "typical" of H. multiforme in that they were non-outcrossing. This included two other stromata from Sweden and a stroma (AB) from a tree only 8 m away from log AA, in Ashclyst Forest. Pairings between genotypes from the Swedish stromata resulted in incompatible interaction types, as did most of those between genotypes from the two Ashclyst Forest samples. However, the log AB genotype intermingled with wood genotype 2 of log AA, with pd ascospore strains and one genotype (of which three out of eight ascospore strains corresponded - see above) from "outcrossing" pc.

Occasionally the same genotype occurred in more than one log within other sites (where only non-outcrossing genotypes were found). For example at Conkwell Wood, genotype 1 was found in two standing trees separated by 10.67 m (Figure 5.10). At Savernake Forest however, the occurrence of genotype 3 in two birch logs separated by only 0.91 m, suggests that these logs were probably once part of the same tree (Figure 5.11). At a third site, Venbridge Wood, the four genotypes found were restricted to individual trees (Figure 5.12).

The occurrence of the same genotype at the same site in different trees may be more common than the data here suggest, as the number of samples collected from each site was small. It seems unlikely however that the same genotype would exist in separate geographic locations. This is because when 47 genotypes from 13 sites were paired in culture they always produced incompatible interaction types (see Chapter 4).

"Hypoxylon purpureum"

Single ascospore strains isolated from the same perithecium on each of four samples from different sites were all somatically compatible when paired. Pairings between wood-derived strains from different cross-sections of the same log also resulted in intermingling mycelia, indicating that single genotypes (with respect to somatic incompatibility loci) occupied considerable volumes of wood (Table 5.7). A genotype from log 2Y for example extended for 3.36 m in a decay column, at least half the cross-sectional area of a beech (Fagus sylvatica) trunk from Friary Woods. This trunk supported H. nummularium perithecial stromata on the outside at felling. A year after felling (and isolation of "H. purpureum" from the central wood cylinder), perithecial stromata of "H. purpureum" developed on the cut surfaces. Another "H. purpureum" genotype stretched for 2.55 m in a beech log (7) from Bath University campus (Wolstenholme, 1986). "Hypoxylon purpureum" was also isolated from silver birch (Betula pendula) wood (log S - see Figure 5.6) bearing H. multiforme stromata, and here it appeared to be confined to a small region of two adjacent decay

zones in one cross-section, H. nummularium and H. multiforme occurring in other zones of the same section.

Pairings between wood and/or ascospore strains from different sources resulted in narrow band, that is somatically incompatible interactions, suggesting that, like H. multiforme, the population is not a single clone.

Rosellinia desmazieresii

An initial sample of R. desmazieresii yielded single ascospore strains that were all somatically compatible (that is intermingled) when paired. Six of a subsequent collection of nine perithecial samples (ascospores from three repeatedly failed to germinate), mapped in the field to show their positions relative to one another, also yielded this result. Three of these samples were taken from a single ring of dead (at the centre) and dying (at the periphery) creeping willow (Salix repens). Strains from these perithecia were somatically compatible, so that a single genotype appeared to be responsible for producing a disease ring 9 m in diameter. Ascospore strains from four other rings (two in the same dune slack - number 31 - and two in an adjacent one - number 29) and wood strains isolated from two different rings in a further slack (number 39) (Figure 5.13) were all somatically incompatible with this genotype, with each other and with a representative isolate of the original sample.

These results indicate that the R. desmazieresii population is

made up of different non-outcrossing genotypes, each capable of individually causing disease and death in a ring of considerable dimensions in the creeping willow sward.

ii. Perithecial production in culture

Single ascospore strains of R. desmazieresii and experimental pairings between ten single ascospore strains of H. mammatum, on CMA both failed to induce perithecial production even after 77 d incubation. The mycelium produced by both these species on this medium was aerially sparse and downy textured. In R. desmazieresii, as on 2% MA, conidia were not evident. Conidia and pseudosclerotial plates (PSPs) were produced in or along many of the H. mammatum interaction zones, which were the same in each pair combination (i.e. narrow line, wide band or bow tie) as they were on 2% MA.

Similarly, after 7 months incubation, paired single ascospore strains of D. concentrica in conical flasks filled with ashwood shavings or newsprint paper were not induced to fruit. Growth in each flask was visible as a downy, white mycelium 7 d after the addition of the conidial suspension. At 14 d small conidial colonies were apparent in the control flasks and on the uncolonized media surrounding plug-inoculated colonies. After 28 d incubation a buff conidial mat had developed over the mycelial surface in all the flasks, and a dark mouse grey to fuscous black pigment became visible in the top 2 mm of the media. This appearance was maintained throughout the rest of the incubation period.

Conidiomata, aerial structures bearing conidia and clear liquid droplets at their apices, became apparent in nine (and three of these were self pairings) of the 55 wood flasks in which isolates were paired and incubated for 40 d. Exposure to 4°C for 3 d had no obvious effect.

5.4 Discussion

The distribution and variation of genotypes in populations of xylariaceous fungi was found to differ between species. In outcrossing (heteromictic) species, where the variation between ascospore strains was shown to be extensive, each genotype was restricted to a single resource unit. These occupied either a considerable volume (sometimes >4 m in length) of a standing tree or attached branch (as in Daldinia concentrica, Hypoxylon nummularium or Hypoxylon rubiginosum) or existed as one of many genotypes each confined to small domains, often restricted to a single log cross-section (as in Hypoxylon fragiforme and Hypoxylon fuscum). "Hypoxylon purpureum", one of the three primarily non-outcrossing species, like D. concentrica, was found to exist as a large single genotype (> 3 m) in standing trunks. Although there was no variation between ascospore strains from the same source, different genotypes were always isolated from separate logs/trees. This indicated that variation did exist in the population. Similarly the population structures of Hypoxylon multiforme and Rosellinia desmazieresii both suggested that although they were principally non-outcrossing species, recombination did occur; a particular genotype was rarely found in more than one resource

unit. That such diversity in population structure exists within a family is not unusual; it is a common feature of certain Angiosperm families including the Rosaceae and the Compositae (Briggs and Walters, 1984).

A notable feature was that in all the species studied, with the exception of H. multiforme, wood-derived strains from different trees/logs were always somatically incompatible, that is novel genotypes were always recovered, suggesting that the infective agents were probably ascospores. It is unlikely that conidia were the agents of infection for, if they were, at least a small proportion of trees at the same site would be expected to be occupied by the same genotype. Even in H. multiforme, the diversity of genotypes just from one site, indicates a dominance of the sexual mode of reproduction in the life cycle. Only twice was a particular genotype of this species recovered from more than one log, and because H. multiforme is primarily non-outcrossing, these may have arisen from ascospores from the same perithecium.

The present investigation suggests that there may be a relationship between the volume of wood occupied by individual genotypes of a species and the size of stromata produced. For example it is interesting that H. nummularium, H. rubiginosum and "H. purpureum" which form extensive effuse stromata and D. concentrica with its relatively large semi-globose stroma, often exist as single genotypes with extensive domains. By contrast the numerous genotypes of H. fuscum and H. fragiforme found in a small

volume of wood, produce a profusion of smaller hemispherical stromata. Such a correlation between fruit body size and occupied domain has been proposed for wood-rotting Basidiomycotina (Rayner et al., 1984). For example, Piptoporus betulinus forms large brackets and usually exists as a single extensive genotype within a tree, whilst Coriolus versicolor is often present as numerous genotypes in cut timber and forms many small sporophores.

The large volume of wood in standing trees or attached branches occupied by individual genotypes of D. concentrica, H. nummularium, H. rubiginosum or "H. purpureum", seems to be inconsistent with the mycelial extension rates of these fungi in culture. Close examination of the wood often revealed no obvious colonization courts, such as wounds or branch stubs. These features suggest that the fungi perhaps gain access to the intact water-filled sapwood via natural discontinuities in the bark (e.g. lenticels). Here they may establish sparsely, possibly as propagules which become disseminated in the sapstream. Later, for example, following alteration of environmental factors that affect the tree, resulting in the alleviation of the microenvironmental conditions which are unfavourable to filamentous growth, the propagules may revert to mycelium, rapidly colonizing extensive volumes of wood. That this latent invasion, a concept first proposed by Boddy and Rayner (1983), may be the colonization strategy adopted by these fungi, is supported by the rapid appearance of stromata frequently observed on trunks and branches following drought or mechanical injury.

The detailed study of a 12 m long ash trunk, log VII, colonized by D. concentrica revealed that, at least for this species, a trunk or log was not always occupied by a limited number of genotypes. A possible explanation for the distribution of the 30 genotypes isolated from this log is that the trunk may have been colonized in two stages. The first stage may have been prior to the death of the tree. This perhaps led to a few genotypes colonizing considerable volumes of wood, by latent invasion, and coming to occupy separate decay columns clearly delimited by interactive zone lines. Immediately following the death of the tree, the second phase of colonization may have occurred. Here several ascospores may have gained access to the centre of the trunk (for example section KL) possibly through wounds created when the tree fell as a result of loss of anchorage through root decay (the tree was found in a semi-fallen position caught by neighbouring trees). The absence of well-defined interaction zone lines between the genotypes in this section, may be a result of the juvenile state their mycelia may have been in when they came into contact. Like mycelia of freshly obtained ascospore strains in culture, they may have interacted in the wood, producing bow-tie and hour-glass reactions (i.e. those involving considerable non-self acceptance - access migration - and limited rejection - see Chapter 4, Section 4.4).

The results obtained for log VII are similar to those reported by Adams (1980) for the birch polypore Piptoporus betulinus. Daldinia appears to have two features in common with P. betulinus. Firstly the same genotype was never obtained for more than one

tree, indicating that the infective agents were ascospores and basidiospores respectively. Secondly, the number of genotypes found in windthrown trunks associated with Armillaria infection was far greater than the number found in standing trunks and attached branches. In 25 trees sampled for Piptoporus the only ones containing more than two genotypes were those infected with Armillaria - one of these contained at least 19 distinct dikaryons within a 1 m section of trunk. These observations suggest that,in standing trunks, opportunities for infection are limited. Hence, if an ascospore has gained access, once the stressful conditions in the tree are relieved (e.g. by drought or light suppression) it can rapidly establish an extensive domain with little or no competition. By contrast, root infection of an otherwise healthy tree predisposes it to multiple infections, whilst still maintaining stressful microenvironmental conditions preventing wholesale infection by saprotrophic competitors. An important distinction however between Daldinia and Piptoporus, is that whilst the former is host-selective, occurring ubiquitously on moribund ash (Fraxinus excelsior) but also on a variety of other broadleaved trees (Whalley and Watling, 1980, 1982), the latter is host-specific, as it only attacks birch (Betula pendula).

Hypoxylon fuscum and H. fragiforme appear to have adopted a similar latent invasion colonization strategy to that described for the species above. However, instead of establishment being effected from within the xylem (presumably a rare event) resulting in a limited number of extensive genotypes, the existence of several

genotypes with relatively small domains indicates that establishment may have occurred from bases in the bark (where opportunities for establishment are increased) (Rayner and Boddy, in press). Support for this comes from examination of wood infected by these Hypoxylon species which reveals no obvious colonization courts. It is reasonable to suggest that numerous genetically different propagules (ascospores) may gain access to the bark of an unstressed tree, and here suitably specialized fungi may be capable of limited saprotrophic survival (Cooke and Rayner, 1984). Once the hostile microenvironmental conditions in the unstressed tree (that restrict extensive mycelial growth) are relieved, these fungi exploit their position by rapidly colonizing the wood. As this occurs from several individual foci (arising from genetically different propagules) the result is a mosaic of overlapping domains. Other species, including Ophiostoma ulmi (Brasier, 1984) and Xylaria hypoxylon (A.D.M. Rayner, pers. comm.) also appear to effect establishment from bases within the bark.

The observed distribution of genotypes within a resource unit suggests that once in the wood H. fuscum and H. fragiforme may behave differently. The occasional H. fuscum genotype occupied a large volume of wood (e.g. log VB). This and the absence of interactive zone lines between some genotypes (where they may have been irretrievably intermixed so that they could not form discrete decay columns), indicate that sometimes this species is distributed in the sapstream, as described above for D. concentrica. It is perhaps the ease of entry by ascospores via establishment in the

bark that results in several genotypes which must compete with one another to capture resources. By contrast, H. fragiforme genotypes were almost always restricted to small domains in a single log cross-section, suggesting that perhaps this species, once in the wood, is not disseminated in the sapstream. It may be that germinating ascospores in bark develop in a restricted mode of growth as described in Chapter 3 (Section 3.3, ii, (a)). Indeed many of the isolates from wood were of the restricted (R) mycelial type. The occurrence of H. fragiforme in sectioned and separately incubated lengths cut from healthy beech (Fagus sylvatica) branches which were exposed to different drying regimes (L. Boddy and I.H Chapela, pers. comm.) provides evidence for a latent invasion strategy in this fungus. In addition, this study indicated that H. fragiforme may also establish from within the xylem as the fungus in the wood was not connected to the bark or cut ends of logs. A latent invasion strategy in H. fragiforme is supported by reports that it exists as an endophyte present without causing symptoms of infection in gorse (Ulex europaeus and Ulex gallii) (Fisher, Anson and Petrini, 1986).

The isolated genotypes of H. nummularium and "H. purpureum" occupying small domains of wood in the centre of logs bearing stromata of other xylariaceous species, may also be a result of the chance entry of an ascospore. Possibly the microenvironmental conditions in the particular tree species do not favour development of mycelia or propagules of these fungi, and so they develop sparsely and are readily surrounded by other xylariaceous species

better adapted to tolerate these particular conditions.

The absence of variation between ascospore strains isolated from the same perithecium in H. multiforme, "H. purpureum" and R. desmazieresii indicates that these species are homomictic (i.e. the haploid homokaryon is self-fertile) or apomictic (i.e. sexual fusion is absent from the formation of ascospores). Outcrossing however can occur as they are ascomycetes with sex organs (Rogers, 1979a). Heterokaryosis is restricted to these structures, unlike Basidiomycotina where outcrossing is dependent on the formation of a heterokaryotic mycelium, which in homomictic or apomictic species is prevented by somatic incompatibility (Rayner and Boddy, 1986).

The detailed examination of log AA seemed to suggest that in H. multiforme a genotype can be both self-fertile (or that ascospores can form without sexual fusion) and able to be fertilized by other genotypes, resulting in adjacent perithecia on the same stroma producing clonal and variable progeny respectively. This assumes that, as in D. concentrica, the stroma was produced by the genotype occupying the wood in the corresponding decay column. In H. multiforme it is almost impossible to show this, as it depends on being certain that a "stromatal isolate" is obtained from stromatal tissue alone. As the stromata of this species are so small, it is difficult to ensure that such tissue is free of ascospores. To circumvent this problem perhaps a stromatal isolate should have been prepared from a stroma prior to ascospore formation.

That outcrossing perithecia were found twice on log AA and once on a Swedish stroma, demonstrates that outcrossing in H. multiforme is not a rare event. It may be that outcrossing is favoured if the opportunity for it arises (presumably there are more opportunities where three genotypes exist together in a small volume of wood such as log AA, than where only one genotype occupies a log). However perhaps homomixis or apomixis can occur if the opportunity for outcrossing does not arise. Hence the occasional occurrence of outcrossing may generate variation in an otherwise predominantly non-outcrossing population, so that the latter is divided into numerous somatically incompatible clonal sub-populations.

The occurrence of one genotype in three out of eight of the progeny of outcrossing perithecium pc, which corresponded to one of the genotypes in the wood and the genotype from log AB, indicates that in H. multiforme a few predominant genotypes may exist. However, it is unfortunate that a more detailed study of the distribution of genotypes from one geographical location could not have been achieved. This would have demonstrated unequivocally the frequency with which particular genotypes appear in different resource units. Further, if particular genotypes had predominated, it would have shown whether they were found outside that area - a situation which occurs in Ophiostoma ulmi (Brasier, 1984) and Cryptostroma corticale (Bevercombe and Rayner, 1984). The sites where H. multiforme was collected appeared to have only a limited number of infected trees, although with its almost ubiquitous occurrence on birch (Betula spp.), a site where there is considerable infection must exist.

Similarly, with respect to R. desmazieresii the preliminary investigation at Ainsdale invites a more extensive study. It is particularly interesting to speculate on how such variation arose between the dead and dying creeping willow (Salix repens) rings, when the ascospore progeny from individual perithecia were always genetically identical with respect to somatic incompatibility loci. It may be that variation is generated in the same way as in H. multiforme, that is by the occasional occurrence of outcrossing.

The failure of H. mammatum and R. desmazieresii to yield perithecia on CMA may imply that these xylariaceous species are more exacting in their requirements for fruiting than Nectria species, that apparently fruit abundantly on this medium (Ian Parrett, pers. comm.) This view may be supported by the occurrence of Nectria species on bark of various trees, in contrast to the host selectivity of these Xylariaceae. Hypoxylon mammatum is considered to occur commonly on poplar (Populus) species although it is found on other broadleaved trees (Manion and Griffin, 1986) and R. desmazieresii occurs on creeping willow (Salix repens) (Barrett and Payne, 1982). Host selectivity is discussed further in Chapter 8.

To conclude, the observed population structure of xylariaceous species studied here appears to be in marked contrast to population studies of other Ascomycotina. Even in the principally non-outcrossing Xylariaceae, genotypes were rarely collected that belonged to the same somatic compatibility group. By contrast studies of Cryphonectria parasitica (Anagnostakis, 1984b),

Ophiostoma ulmi (Brasier, 1984) and Cryptostroma corticale (Bevercombe and Rayner, 1984) all resulted in the recovery of several genotypes, often from quite distant geographic locations, that belong to the same somatic (vegetative) compatibility group. It may be that the examination of several species, rather than just one in the present study, resulted in too few samples being collected to show whether repeat genotypes existed in the population. However, it seems more likely that the breeding patterns, that are the source of variation of these primarily saprotrophic* Xylariaceae differs from those of the necrotrophic pathogens mentioned above. Hence these Xylariaceae appear to reproduce principally by sexual outcrossing. By contrast the widespread existence of certain genotypes in the populations of C. parasitica, O. ulmi and C. corticale indicates that these species depend on both sexual and asexual reproduction. A population may be founded by ascospores and then a single successful genotype may become dominant through subsequent asexual propagation - the so-called founder effect.

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* Rosellinia desmazieresii and Hypoxylon mammatum may be exceptions as they may be necrotrophic pathogens, but in this generalization they are disregarded as the survey of both was limited. Further two of the three H. mammatum samples were on willow (Salix spp.) and it is only considered to be a pathogen of poplar (Populus spp.) (Manion and Griffin, 1986).

Table 5.1. *Daldinia concentrica*. The number and extent of genotypes in a given length of wood and the number of stromata produced by each genotype.

Log Code	Type of Wood	Site Code (see Appendix I)	Log Length (m)	Number of isolates		Number of genotypes	Maximum length of a single genotype (m)	Maximum number of genotypes per section	Number of stromata per genotype	Other xylariaceous species isolated (see relevant tables)
				wood	stroma					
I	ash (*Fraxinus excelsior*)	B2	0.27	4	2	1	0.27	1	2	-
II	ash	B2	0.55	14	3	2	0.55	2	3	-
III	ash	B2	0.26	6	3	1	0.26	1	3	-
IV	ash	B2	0.52	15	0	4	0.30	2	0	-
V	ash	B2	0.24	13	5	2	0.24	2	5	-
VI	ash	B2	0.22	16	1	1	0.22	1	1	-
VII	ash	B2	12.485	72	12	30	4.95	14	6,3,2 and 1	-
VIII	ash	B2	0.456	2	7	3	0.304	2	5, 1 and 1	-
1	ash	F	0.10	2	1	1	0.10	1	1	-
2	ash	F	0.22	1	1	1	0.22	1	1	-
3	ash	F	0.17	1	1	1	0.17	1	1	-
4	ash	F	0.30	1	2	1	0.30	1	2	-
R7	ash	A3	2.2	5	0	3	2.2	2	not recorded	-
RF	ash	F	1.35	2(11)	0	1	< 0.04	1	0	*Hypoxylon nummularium* and *H. rubiginosum*

* Figures in brackets refer to the total number of successful isolations of xylariaceous fungi.

Table 5.2. *Hypoxylon rubiginosum*. The number and extent of genotypes in a given length of wood.

Log code	Type of wood	Site code (see Appendix I)	Log length (m)	Number of isolates*	Number of genotypes	Maximum length of a single genotype (m)	Maximum number of genotypes per section	Other xylariaceous species isolated (see relevant tables)
R1	ash (*Fraxinus excelsior*)	A3	0.6	4	1	0.6	1	-
R2	ash	A3	1.8	11	2	1	2	-
R3	ash	A3	1	12	1	1	1	-
R4	ash	A3	0.65	4	1	0.65	1	-
R5	ash	A3	1.2	11	2	< 1	1	-
R6	ash	A3	2	11	1	< 1	1	-
RF	ash	F	1.35	8(11)	2	0.6	1	*Hypoxylon nummularium* and *Daldinia concentrica*

* Figures in brackets refer to the total number of successful isolations of xylariaceous fungi.

Table 5.3. Hypoxylon fragiforme. The number and extent of genotypes in a given length of wood.

Log code	Type of wood	Site code (see Appendix I)	Log length (m)	Number of isolates*	Number of genotypes	Maximum length of a single genotype (m)	Maximum number of genotypes per section	Other xylariaceous species isolated (see relevant tables)
AS1	beech (Fagus sylvatica)	A3	2	14	7	1.5	5	-
AS2	beech	A3	2	18	16	< 0.5	4	-
VW	beech	V	10	53	51	< 4	13	-
T2	beech	C4	1	7(15)	7	< 0.5	4	Hypoxylon nummularium

* Figures in brackets refer to the total number of successful isolations of xylariaceous fungi.

Table 5.4. Hypoxylon fuscum. The number and extent of genotypes in a given length of wood.

Log code	Type of wood	Site code (see Appendix I)	Log length (m)	Number of isolates*	Number of genotypes	Maximum length of a single genotype (m)	Maximum number of genotypes per section	Other xylariaceous species isolated (see relevant tables)
12	hazel (Corylus avellana)	F	0.97	8	8	< 0.5	5	-
14	hazel	F	2.2	9	9	< 0.83	3	-
15	hazel	F	1.1	18	16	0.78	9	-
CC1	hazel	C1	1.4	2	1	0.5	1	-
CC2	hazel	C1	1.5	5	4	0.5	3	-
M1	hazel	M	0.9	6	3	0.3	1	-
M2	hazel	M	0.55	4	5	< 0.3	2	-
M3	hazel	M	0.68	4	3	0.3	3	-
M4	hazel	M	0.8	1	1	< 0.4	1	-
SB1	hazel	S3	1.2	3	1	0.6	1	-
SB2	hazel	S3	1.10	6	4	0.7	2	-
VB	birch (Betula sp.)	V	2.25	12(16)	6	1.7	3	Hypoxylon nummularium
F4	hazel	F	1.93	11(12)	5	0.8	2	H. nummularium
F5	hazel	F	3.12	6(22)	5	<0.5	5	H. nummularium and Hypoxylon multiforme

Table 5.5. *Hypoxylon nummularium*. The number and extent of genotypes in a given length of wood.

Log code	Type of wood	Site code (see Appendix I)	Log length (m)	Number of isolates*	Number of genotypes	Maximum length of a single genotype (m)	Maximum number of genotypes per section	Other xylariaceous species isolated (see relevant tables)
HN2	beech (*Fagus sylvatica*)	F	1.68	7	5	1.3	2	-
HN4	beech	F	0.75	4	3	< 0.35	2	-
HN5	beech	V	0.7	4	3	< 0.7	2	-
HN6	beech	C2	2.3	14	9	0.9	3	-
T2	beech	C4	1	8(15)	6	< 0.5	3	*Hypoxylon fragiforme*
F4	hazel (*Corylus avellana*)	F	1.93	1(12)	1	< 1	1	*Hypoxylon fuscum*
F5	hazel	F	3.12	12(22)	4	< 1	3	*Hypoxylon fuscum* and *Hypoxylon multiforme*
S	silver birch (*Betula pendula*)	F	2.10	4(15)	4	0.2	2	"*Hypoxylon purpureum*" and *H. multiforme*
VB	birch (*Betula* sp.)	V	2.25	4(16)	2	1	1	*H. fuscum*
HM2	hazel	C2	2.45	4(11)	4	< 1	2	*H. multiforme*
RF	ash (*Fraxinus excelsior*)	F	1.35	1(11)	1	< 0.3	1	*Daldinia concentrica* and *Hypoxylon rubiginosum*

* Figures in brackets refer to the total number of successful isolations of xylariaceous fungi.

Table 5.6. *Hypoxylon multiforme*. The number and extent of genotypes in a given length of wood.

Log code	Type of wood	Site code (see Appendix I)	Log length (m)	Number of isolates*	Number of genotypes	Maximum length of a single genotype (m)	Maximum number of genotypes per section	Other xylariaceous species isolated (see relevant tables)
HM1	silver birch (*Betula pendula*)	F	0.55	2	1	0.3	1	-
HM4	silver birch	V	1	6	1	1	1	-
HM5	silver birch	V	1.85	4	1	1	1	-
HM6	silver birch	V	2.8	10	1	2.4	1	-
HM2	hazel (*Corylus avellana*)	C2	2.45	7(11)	2	1.5	2	*Hypoxylon nummularium*
S	silver birch	F	2.10	8(15)	1	1.42	1	*H. nummularium* and "*Hypoxylon purpureum*"
F5	hazel	F	3.12	4(22)	1	2	1	*H. nummularium* and *Hypoxylon fuscum*
AA	silver birch	A3	0.3	5	3	0.206	2	-

* Figures in brackets refer to the total number of successful isolations of xylariaceous fungi.

Table 5.7. "*Hypoxylon purpureum*". The number and extent of genotypes in a given length of wood.

Log code	Type of wood	Site code (see Appendix I)	Log length (m)	Number of isolates*	Number of genotypes	Maximum length of a single genotype (m)	Maximum number of genotypes per section	Other xylariaceous species isolated (see relevant tables)
2Y	beech (*Fagus sylvatica*)	F	7.14	11	1	3.36	1	-
S	silver birch (*Betula pendula*)	F	2.10	3(15)	1	<0.3	1 (2 decay zones)	*Hypoxylon multiforme* and *Hypoxylon nummularium*
7+	beech	B1	2.55	12	1	2.55	1	-

* Figures in brackets refer to the total number of successful isolations of xylariaceous fungi.

+ This result was provided by Ruth Wolstenholme (Wolstenholme, 1986).

Figure 5.1. Distribution of individual genotypes of Daldinia concentrica in log VII

(Bathwick Woods; Fraxinus excelsior).

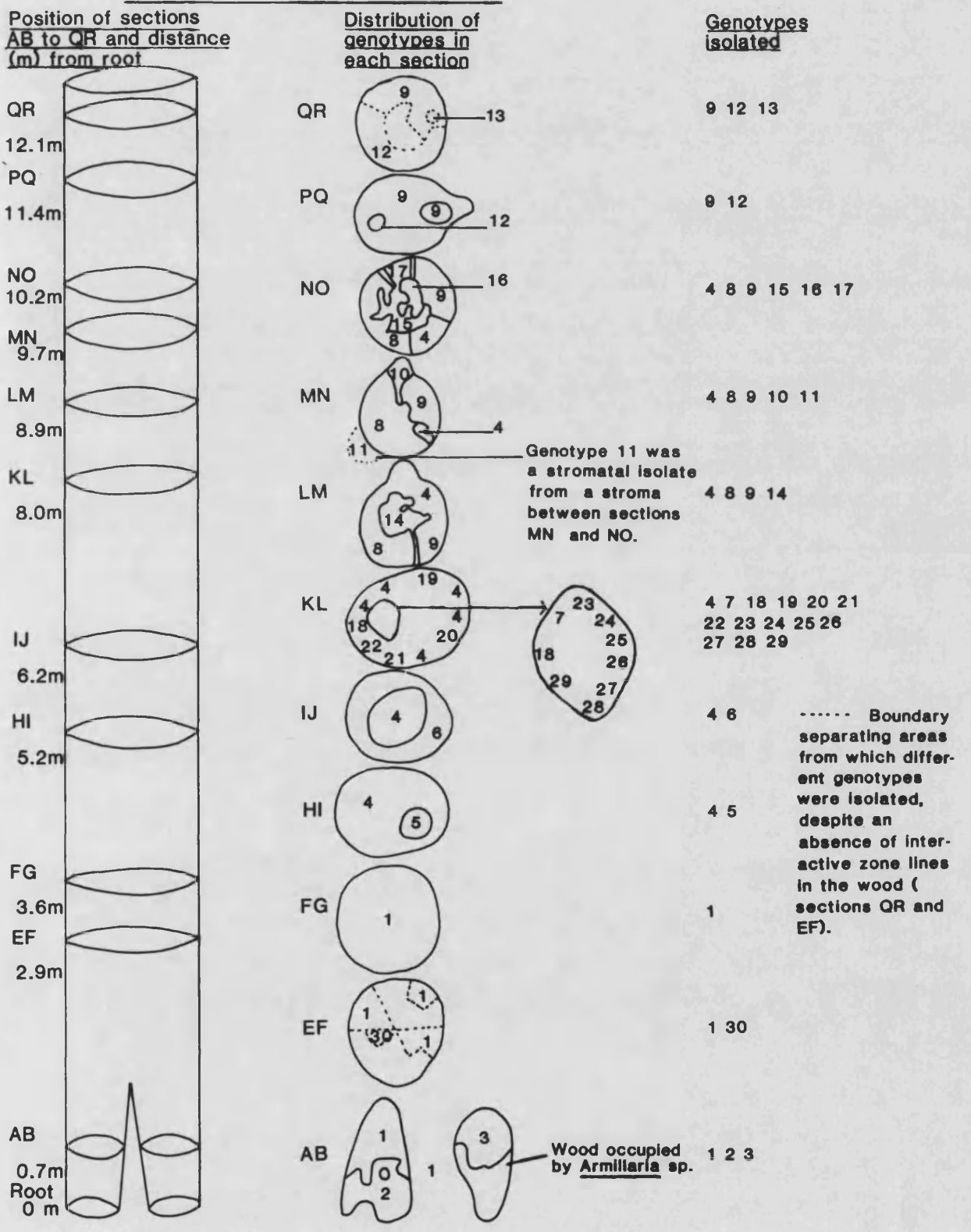
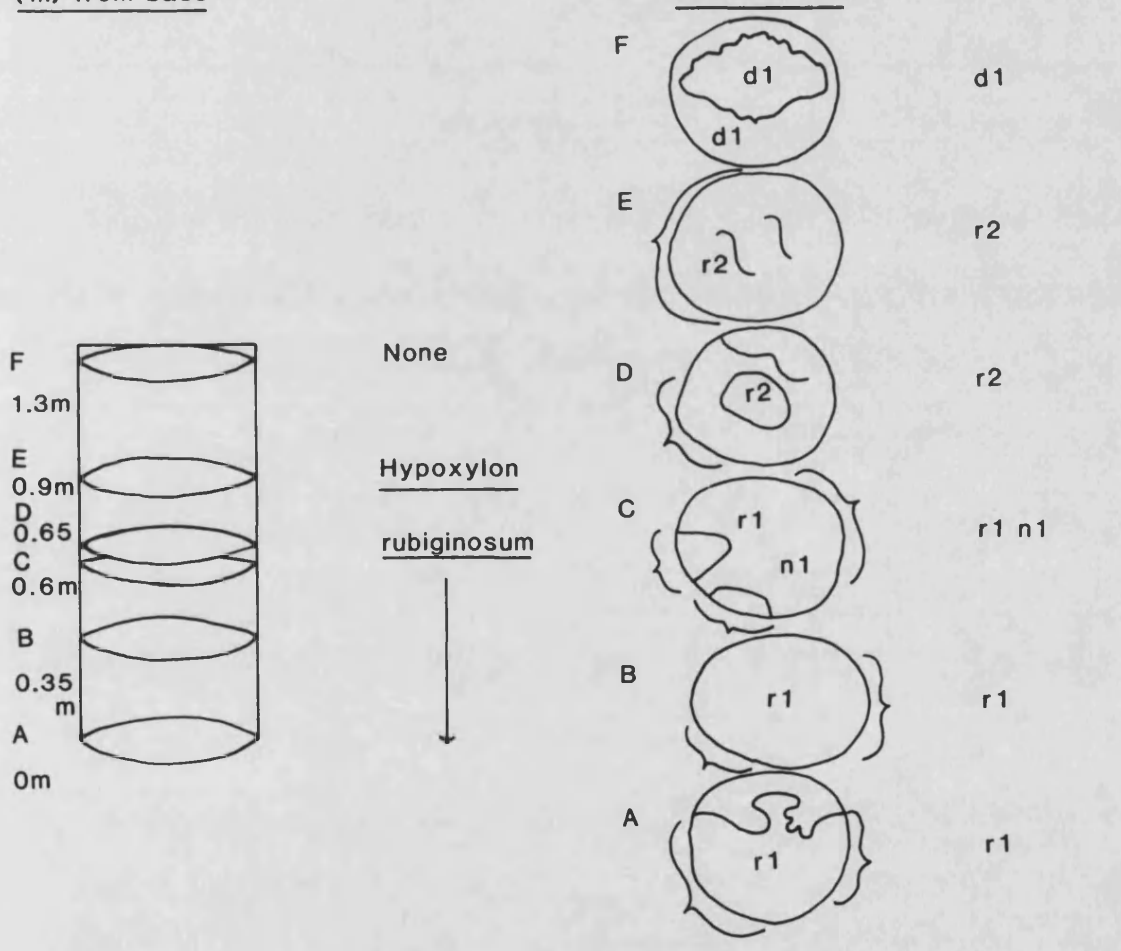


Figure 5.2. Distribution of individual genotypes in log RF

(Friary Wood, Avon ; Fraxinus excelsior)



d denotes Daldinia concentrica, r Hypoxylon rubiginosum ,and n Hypoxylon nummularium.

{ denotes position of stromata

Figure 5.3. Distribution of individual genotypes of Hypoxylon fragiforme in log VW

(Venbridge Wood; Fagus sylvatica)

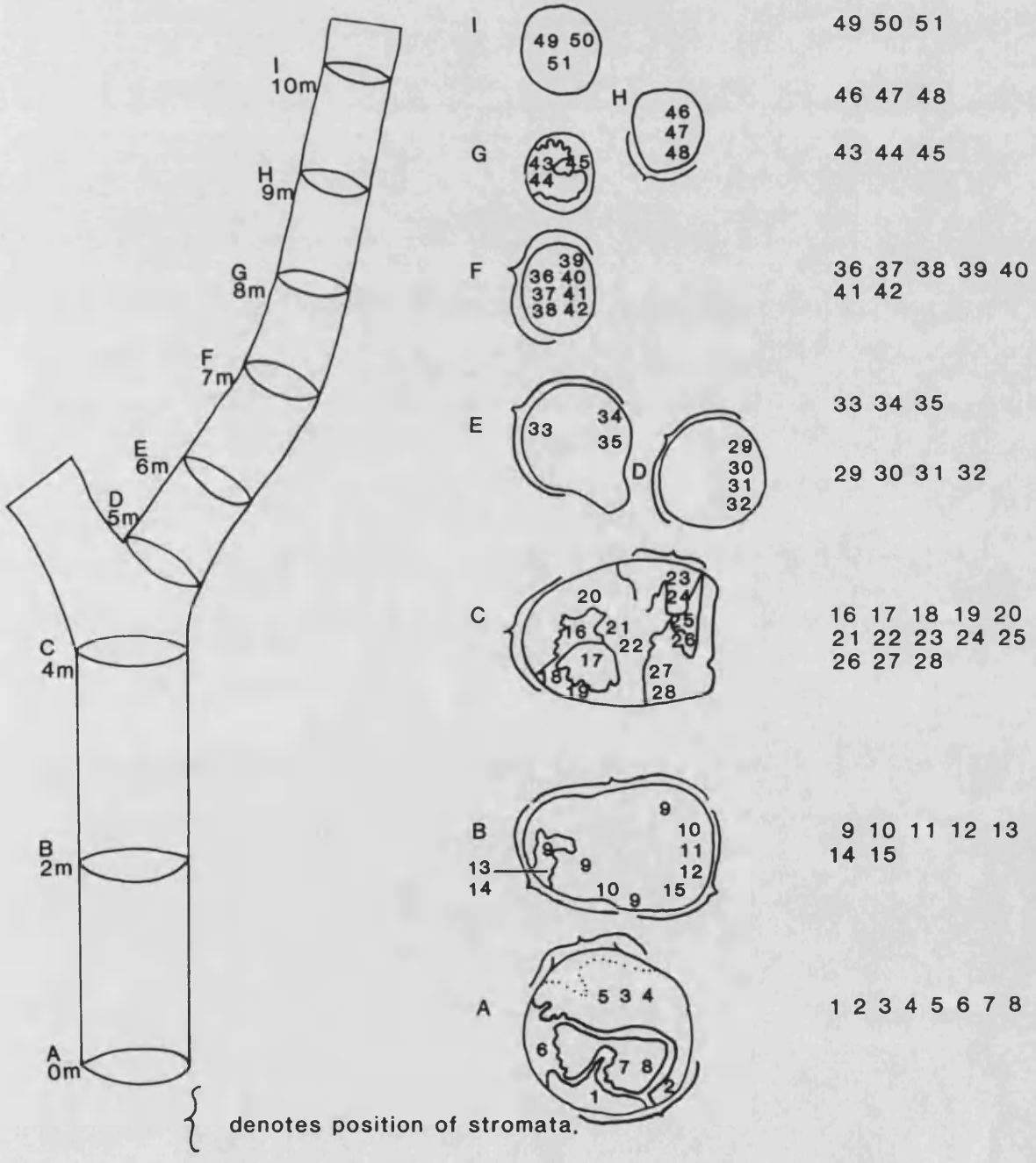
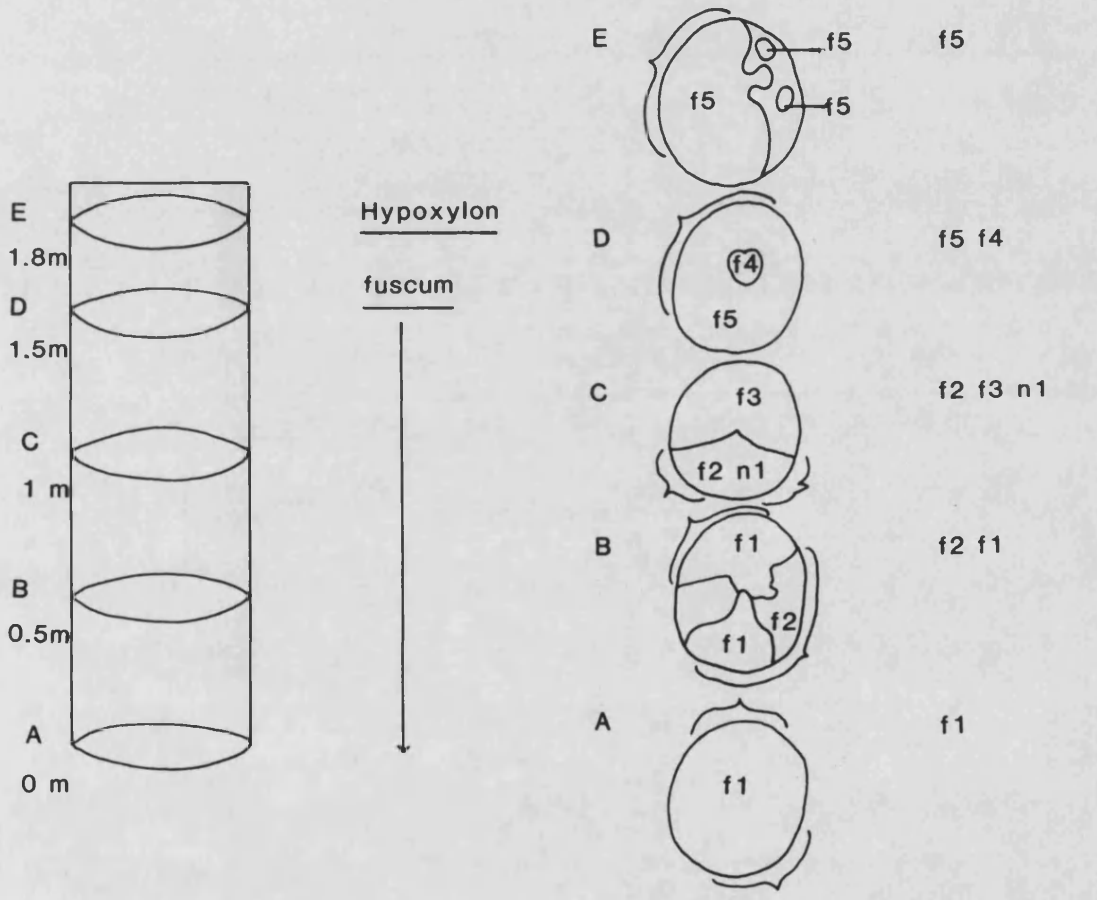


Figure 5.4. Distribution of individual genotypes in log F4

(Friary Wood, Avon; Corylus avellana)

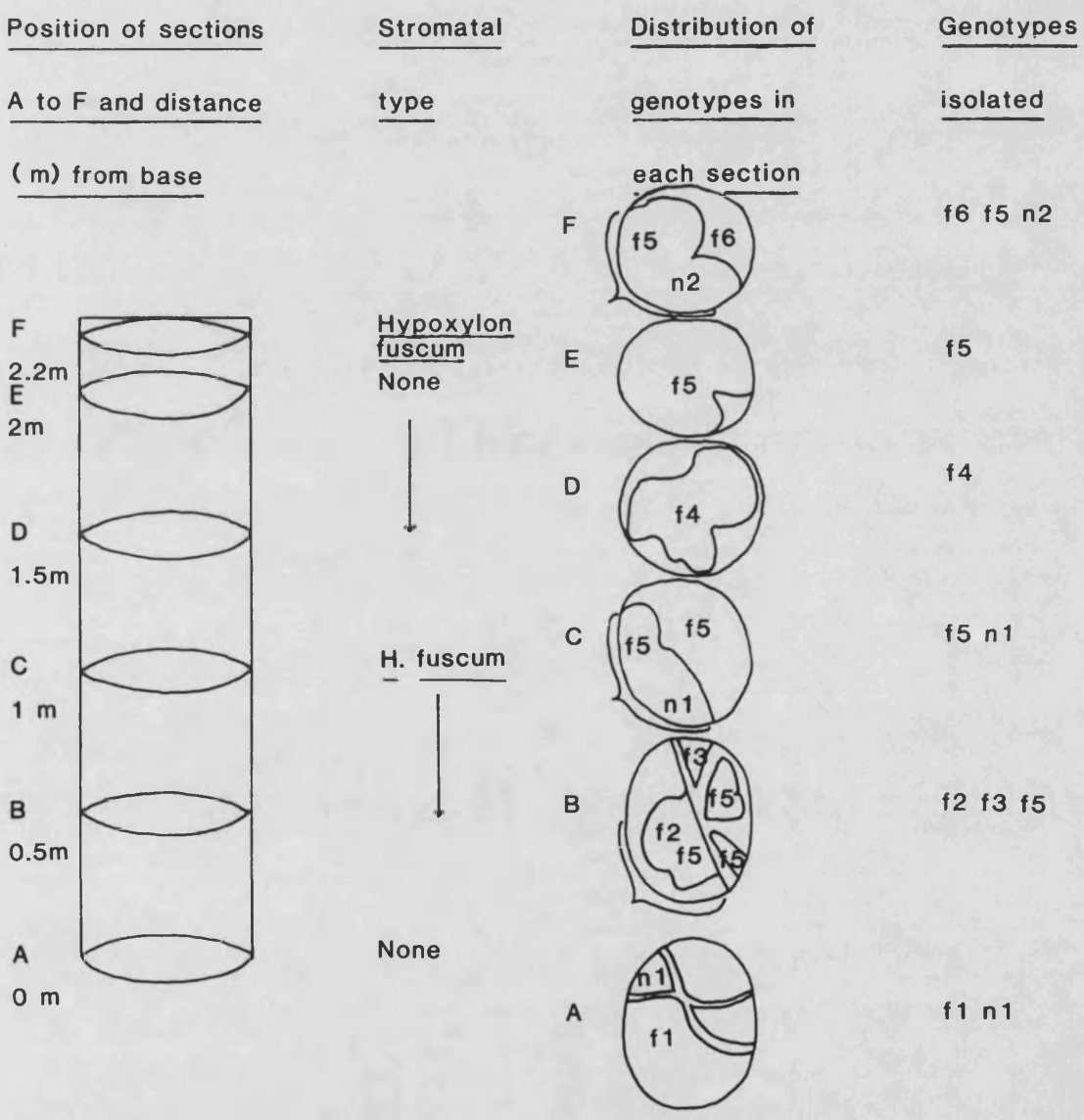


f denotes Hypoxylon fuscum , and n Hypoxylon nummularium

{ denotes position of stromata

Figure 5.5. Distribution of individual genotypes in log VB

(Venbridge Wood; Betula sp)

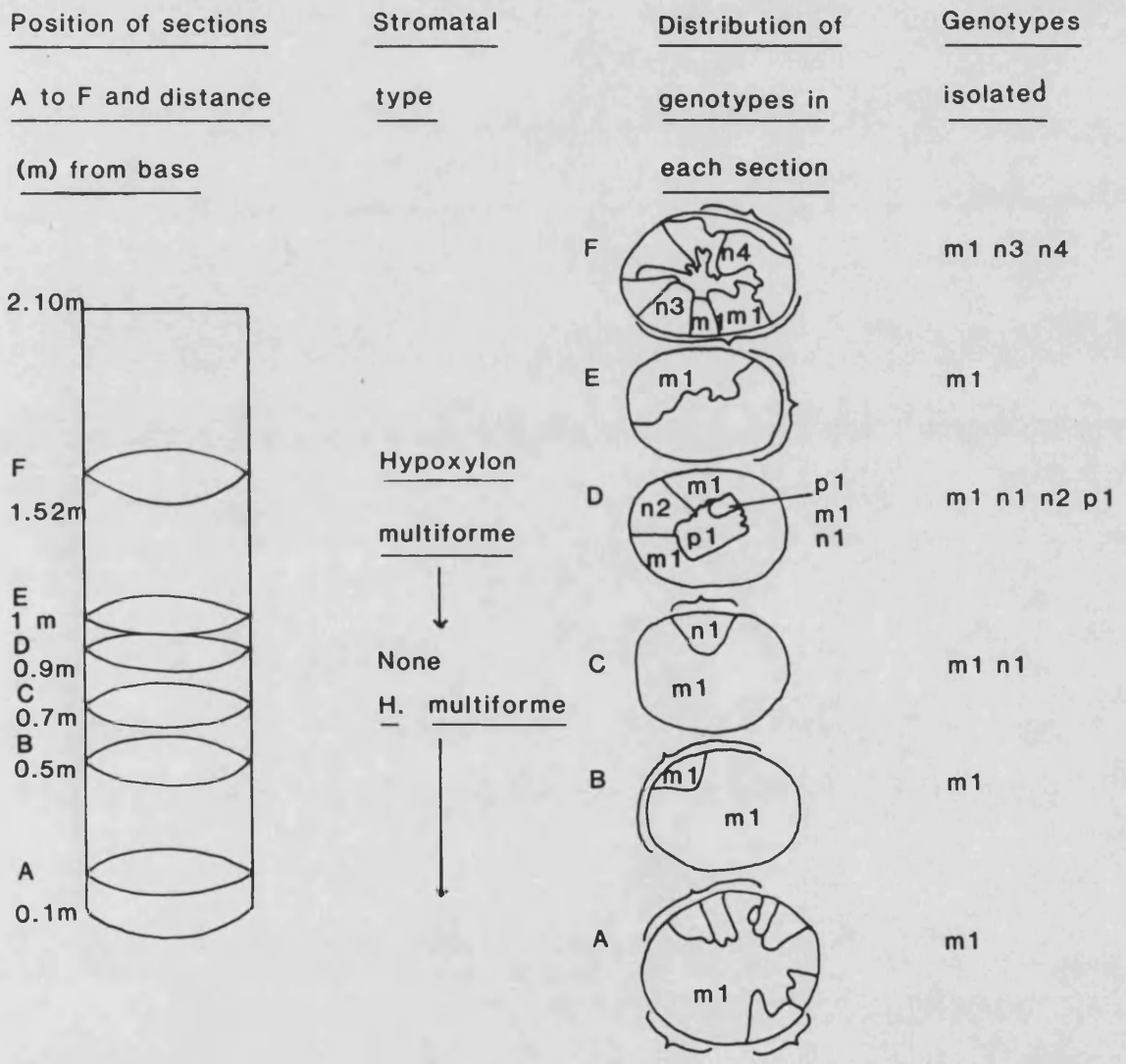


f denotes Hypoxylon fuscum and n Hypoxylon nummularium

{ denotes position of stromata

Figure 5.6. Distribution of individual genotypes in log S

(Friary Wood, Avon; Betula pendula)

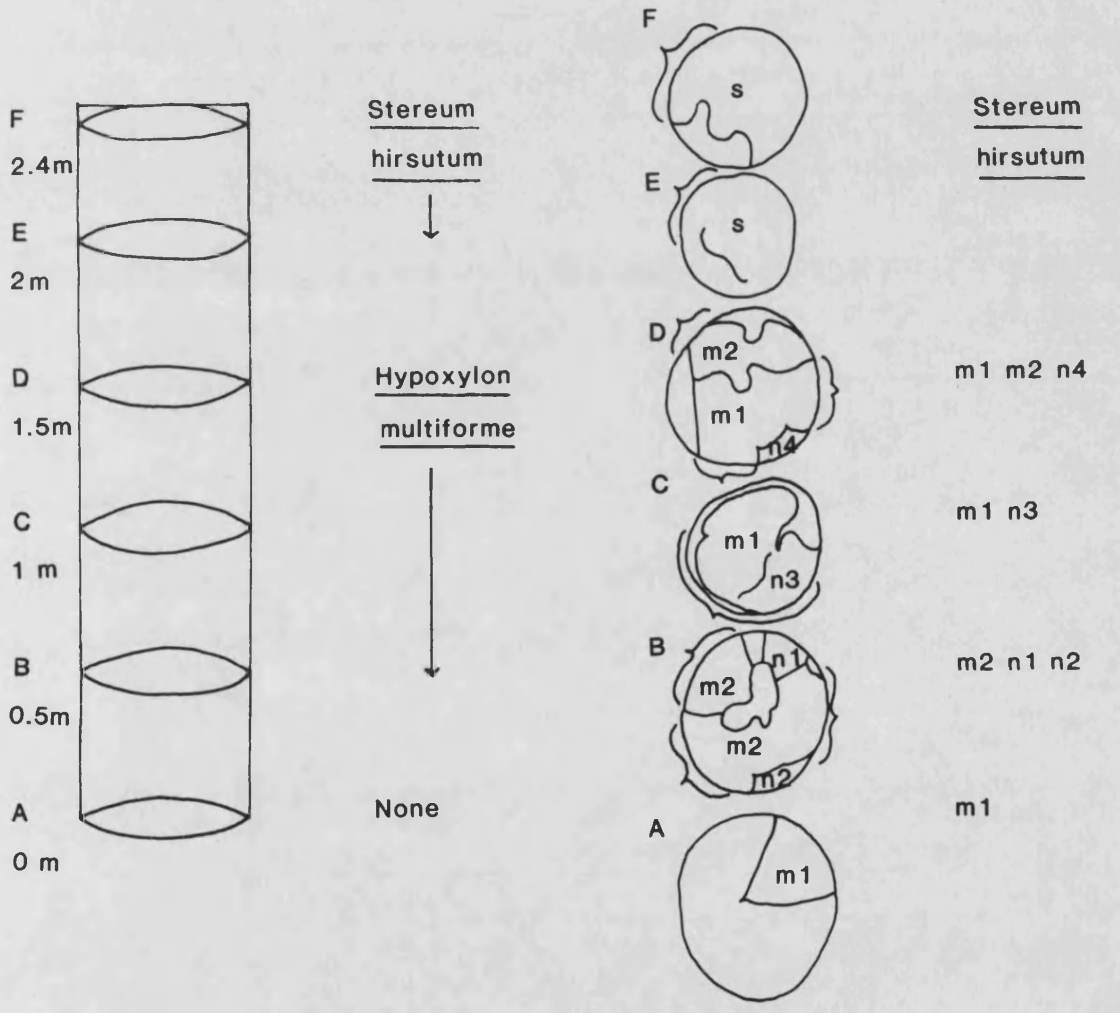


m denotes Hypoxylon multiforme, n Hypoxylon nummularium and p Hypoxylon purpureum.

{ denotes position of stromata

Figure 5.7. Distribution of individual genotypes in log HM2

(Clifford Bridge,Devon; Corylus avellana)

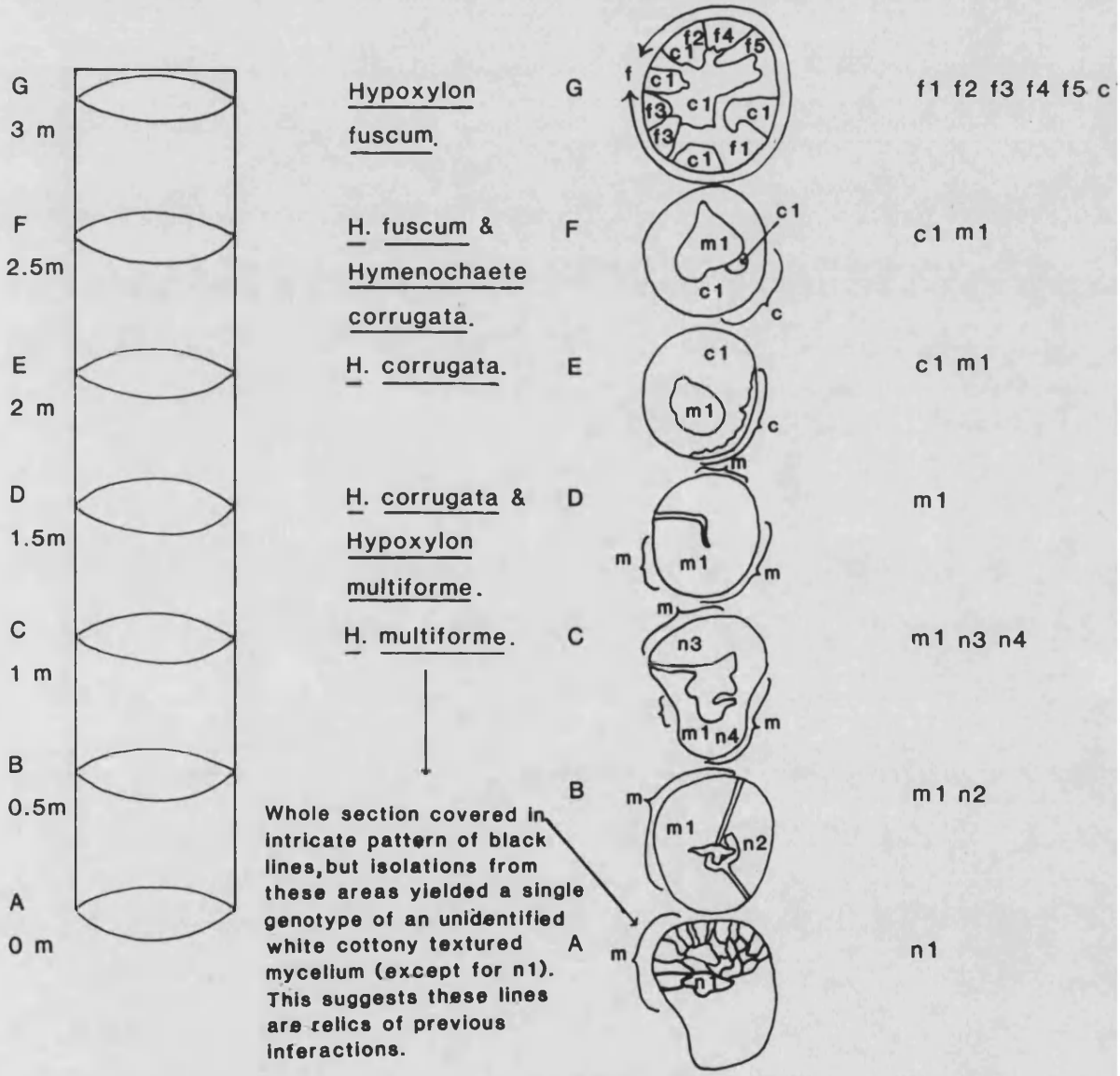


m denotes Hypoxylon multiforme and n Hypoxylon nummularium

{ denotes position of stromata

Figure 5.8. Distribution of individual genotypes in log F5

(Friary Wood, Avon; *Corylus avellana*)



f denotes *Hypoxylon fuscum*, c *Hymenochaete corrugata*, m *Hypoxylon multiforme* and n *Hypoxylon nummularium*.

{ denotes position of stromata

Figure 5.9. Distribution of individual genotypes of Hypoxylon multiforme in log AA (Ashclyst Forest; Betula pendula)

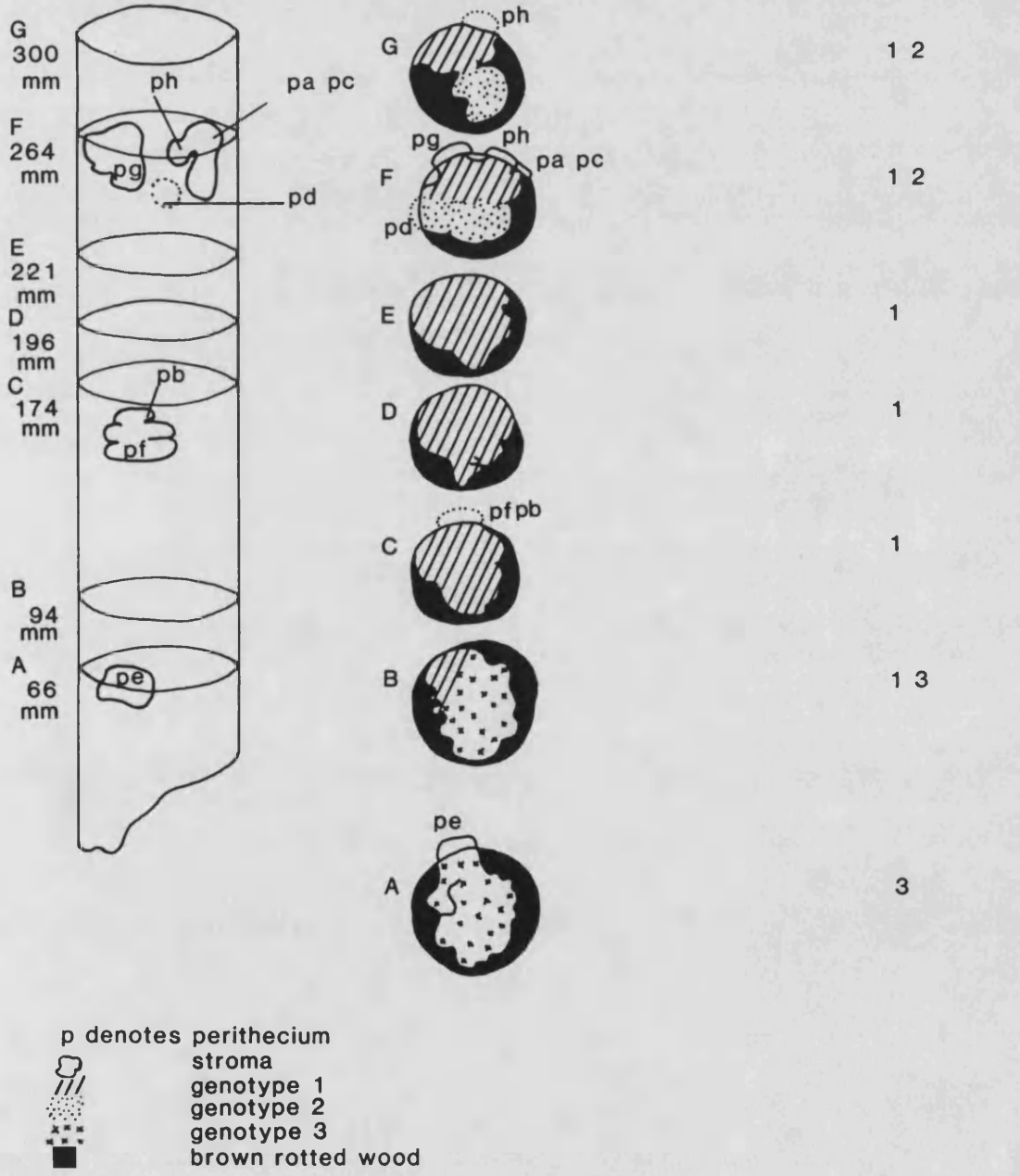


Figure 5.10. Distribution of three Hypoxylon multiforme genotypes in standing alder (Alnus glutinosa) trees in Conkwell Wood, Wiltshire.

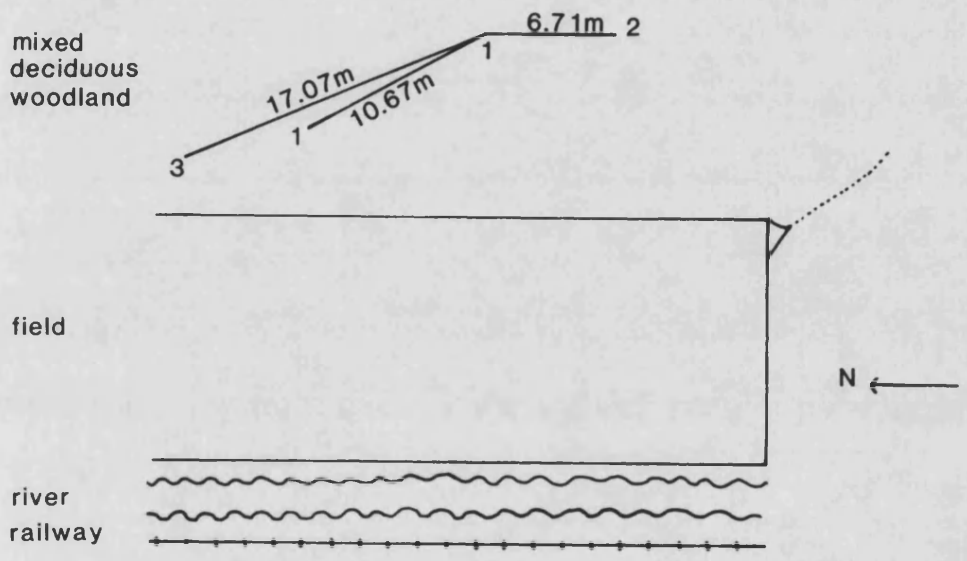


Figure 5.11. Distribution of four Hypoxylon multiforme genotypes in birch (Betula pendula) logs at Savernake Forest, Wiltshire.

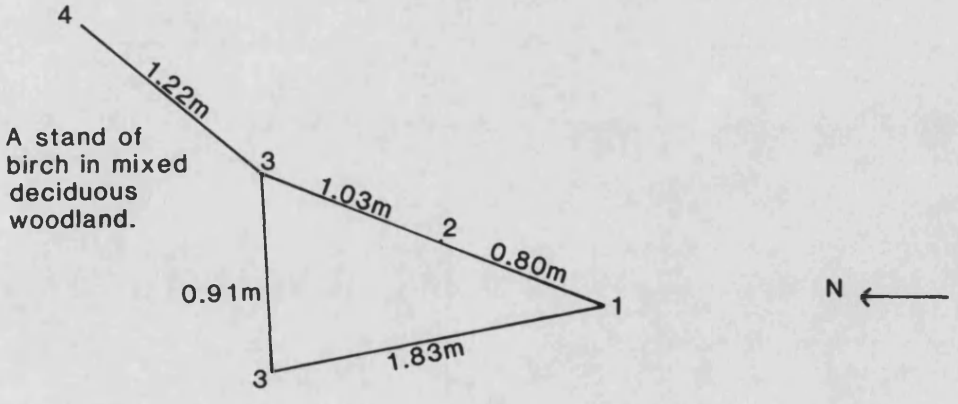
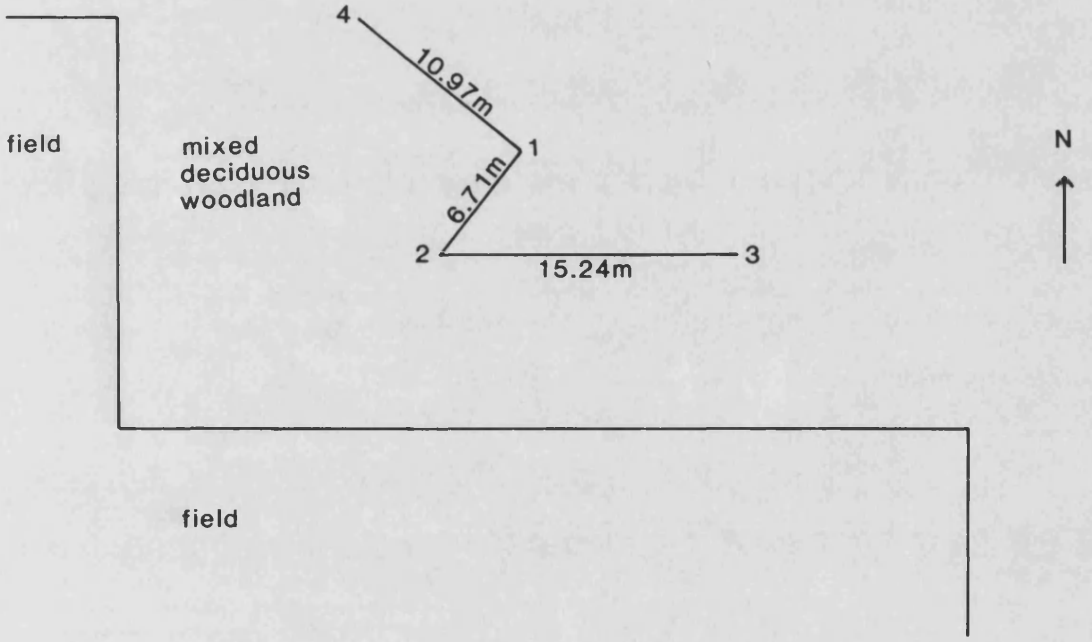


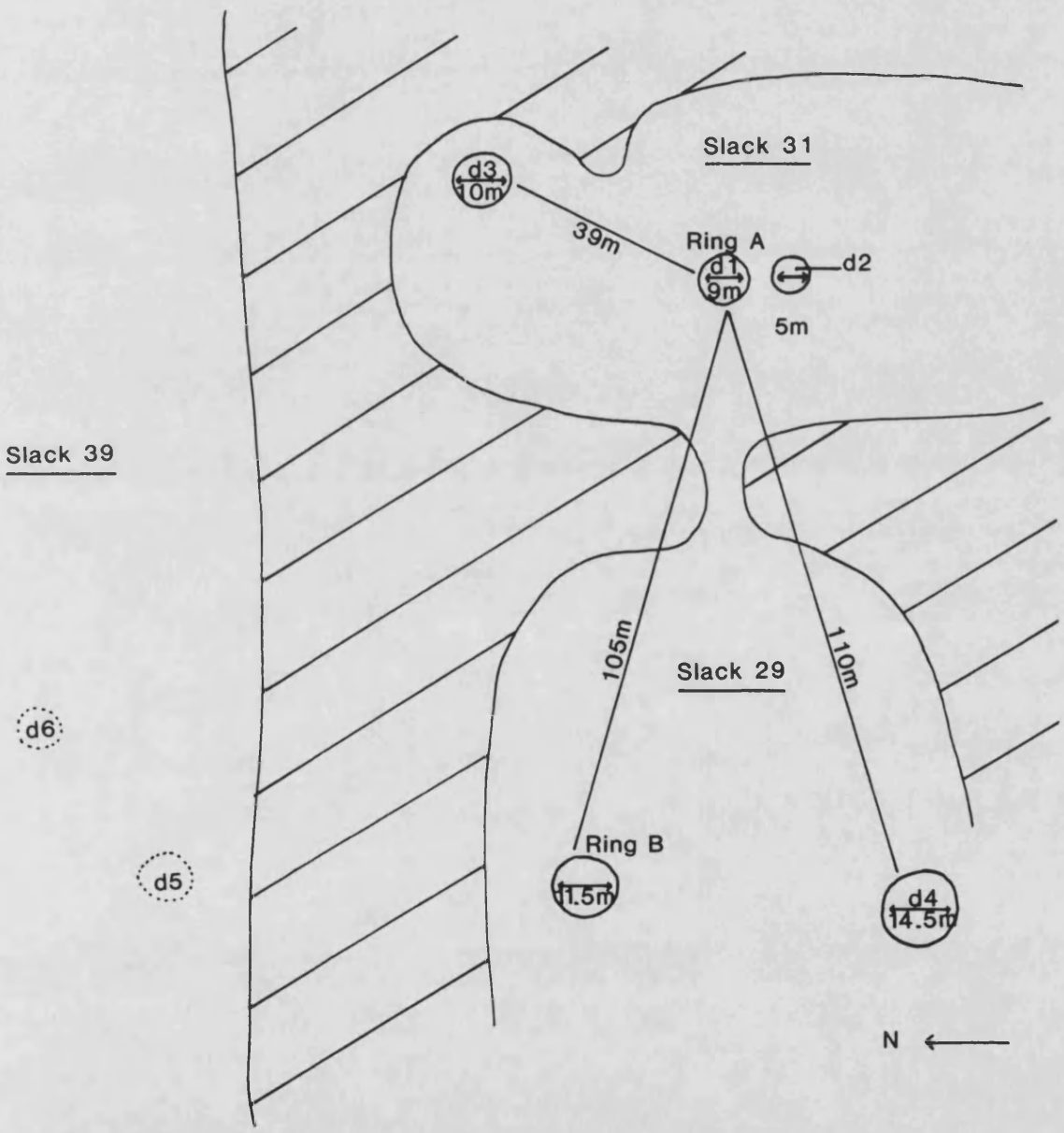
Figure 5.12. Distribution of four Hypoxylon multiforme genotypes in standing birch (Betula pendula) and hazel (Corylus avellana) trees in Venbridge Wood, Devon.



Genotypes 1, 3 and 4 in birch.

Genotype 2 in hazel coppice.

Figure 5.13. Distribution of Rosellinia desmazieresii genotypes in rings of dead and dying Salix repens in three dune slacks at Ainsdale Sand Dunes Nature Reserve.



Ring A — Five perithecial samples were collected at approximately equal distance from one another around the periphery of this ring. Ascospores from two samples failed to germinate, those from the other three were all genotype d1.

Ring B — Ascospores from a sample from this ring failed to germinate.

Corsican pine.

The original sample (from A. Ofong, Oxford University) was somatically incompatible with genotypes d1 to d6 above and was assigned to genotype d7.

CHAPTER 6

INTERSPECIFIC INTERACTIONS AND THEIR ROLE IN DECAY COMMUNITIES CONTAINING XYLARIACEAE

6.1 Introduction

The structure and development of communities in decaying wood embraces interactions within species (such as those described in Chapter 4) and those between species. At the community level it is common to regard species rather than individual genotypes as the basic organizational unit. These species are composed of populations and the outcome of interspecific interactions may not be consistent between different genotypes within such populations or under different conditions. Nonetheless, in general, individual species can be assigned particular behaviour patterns and these can be used to elucidate factors that underlie the stability and change in community composition (Rayner and Boddy, in press).

Interaction types can be simply classified according to a scheme proposed by Cooke and Rayner (1984). Interactions between two species exploiting a common resource may be recognized as being competitive, neutralistic or mutualistic. This depends on whether the outcome is detrimental to either or both participants, detrimental to neither but not beneficial to both, or beneficial to both respectively.

Competitive interactions may be subdivided into two phases, primary resource capture and combat. The former describes the

process of gaining initial access to and influence over a resource. It does not involve contact between participants and depends on, for example, effective dispersal mechanisms and mycelial extension rates. Combat occurs as the domains of different individuals come into proximity following primary resource capture and results from direct physiological challenge.

Truly neutralistic or mutualistic associations in fungi may be common in nature, however there are a few known examples (Cooke and Rayner, 1984; Rayner and Webber, 1984). This may be because interactions rarely occur in the absence of beneficial or detrimental effects, and although benefits may often be available (for example through waste products or exudates of one organism providing a resource for another) they are frequently associated with deleterious side effects (Rayner and Boddy, in press).

Combative fungal interactions are mediated on two distinct levels, that of individual somatic hyphae and mycelia as a whole. Hyphal interactions include hyphal fusion, hyphal interference and parasitism. Mycelial interactions encompass the antagonistic events that lead up to deadlock, in which each participant is mutually excluded from the other's domain, or replacement of one by the other.

In experimental studies using artificial culture the events that precede deadlock or replacement can occur prior to contact, involving, for example, inhibition of colony margin extension

arising from antibiosis. It is more common that obvious interaction between mycelia in culture follows contact. This may include inhibition of extension of one or both colonies, associated with pigment production, lysis and alterations of the mycelium to distinctive morphological modes. The latter may include for example dense mycelial fronts, aggregations into linear organs and pseudo-sclerotial plate production serving to hold or extend domain. Hence the recognition response, as in intraspecific pairings, appears to activate genetic mechanisms that regulate mycelial switching between distinct morphological modes (discussed in Chapter 3).

Combative ability varies between species, so that when a range of fungi are paired together in all combinations, a hierarchy is often evident. Highly combative species may rarely be replaced by others and/or frequently replace many other fungi, whilst less combative species may be poor at attack and defence. However, sometimes a species well down the hierarchy can come out favourably in a confrontation with a species at the top (Rayner and Boddy, in press).

Interaction outcome is known to be influenced by abiotic variables such as water potential and gaseous regimes, although only a few experimental studies into this subject have been made. Despite this, cultural studies of interactions resulting from inter-hyphal or inter-mycelial responses (of the type described above) produced by wood-decay fungi on 2-3% malt extract agar, often correlate almost perfectly with interaction patterns that

occur in naturally decomposing wood (Rayner and Webber, 1984; Rayner and Boddy, in press).

This chapter describes interactions recorded in culture between members of the Xylariaceae and between xylariaceous and other species that were isolated from the same resource. From these observations the possible role that these species play in the structure and development of natural fungal communities are considered.

6.2 Materials and Methods

The procedures used are described in Chapter 2, Sections 2.1 to 2.5. Where appropriate the three-dimensional structure of fungal communities in individual resource units (logs) was analysed according to the methods explained in Chapter 5, Section 5.2, i.

6.3 Results

i. Interactions between xylariaceous species

A well defined zone, usually marked by pigment, was observed at the interaction interface in all pairings between different xylariaceous species. These interactions could be assigned to one of two types, deadlock or unilateral replacement, depending respectively on whether the colonies both retained their original domains, or whether one colony invaded and gained domain from the other.

Most species-combinations always produced consistent deadlock or replacement interactions irrespective of the different genotypes paired. However, different genotype combinations gave variable results in interactions involving Hypoxylon nummularium versus Hypoxylon multiforme or Hypoxylon rubiginosum.

(a) Deadlock

Deadlock interactions were observed between the three Hypoxylon species isolated from beech (Fagus sylvatica), (H. nummularium, H. fragiforme and "H. purpureum"). They were also observed between H. multiforme versus Hypoxylon fuscum (isolated together from hazel - Corylus avellana) or "H. purpureum", as well as between certain genotypes of the other species mentioned above (Table 6.1). The interaction interface was usually marked by a zone of morphologically distinctive mycelium in only one of the two colonies. Here the mycelial characters of the relevant species were altered, so that the hyphae were appressed to the agar surface (aerial hyphae were sparse) and in some cases clearly lysed. Such zones were broad, a minimum of 5 mm and sometimes exceeding 10 mm in width, and when examined from below were usually intensely pigmented (Figure 6.1 A, B, C, D).

Where species with dissimilar linear extension rates were paired, these differences resulted in the mycelium with the slower rate being surrounded by that with the faster rate, so that the latter was spatially dominant. Nonetheless the interaction was recorded as deadlock since neither species was capable of growing

into medium already occupied by the other. However, in H. fragiforme versus "H. purpureum" and H. nummularium versus H. rubiginosum the mycelia with the slower linear extension rate ("H. purpureum" and H. rubiginosum respectively) appeared to suppress or inhibit mycelial extension of the other participant, reducing its ability to establish on the uncolonized agar. Intensely coloured pigment was produced by the suppressed mycelium in both instances (Figure 6.1 E, F, G, H) and where "H. purpureum" was involved the bitter-sweet smell usually associated with this species intensified. Ultimately (> 21 d incubation) linear extension of the H. fragiforme colony was halted, but the "H. purpureum" mycelium continued to colonize fresh agar along its margins distal to H. fragiforme. By contrast extension of the H. nummularium mycelium was only slowed, so that eventually since it still possessed a faster extension rate than H. rubiginosum it encircled the mycelium of the latter species.

(b) Unilateral replacement

Unilateral replacement reactions occurred in all pairings in which Daldinia concentrica was a participant. They also occurred in pairings involving H. nummularium versus H. fuscum, H. rubiginosum or H. multiforme and between H. multiforme versus H. mammatum or "H. purpureum" (Table 6.2).

Colonies of H. fragiforme, H. rubiginosum and H. nummularium were entirely, or almost entirely, replaced by a front of white, silky textured aerial D. concentrica mycelium, in which the

olivaceous flecks characteristic of this species were absent (Figure 6.2 A, B). The domain previously occupied by the invaded colony was only evident underneath by the pigment (usually sepia) it produced and often along the original interaction interface (i.e. where the two colonies first met) there was a diffuse band of variable width (5–20 mm) of amber D. concentrica pigment.

Less extensive replacement of one mycelium by another was observed in other species combinations. Here, rather than an entire colony, only a band (5–20 mm) of mycelium was involved and encroachment was halted along a line of pigment, visible from below, produced by the invaded colony (Figure 6.2 C, D, E, F). As with D. concentrica replacement was mediated by the production of a mycelial front of densely-packed hyphae which did not appear to be preceded by lysis, but merely seemed to "smother" the encroached colony. Hypoxylon nummularium was always replaced by another species in this manner, except for a proportion of pairings with H. fuscum in which replacement by the latter was preceded by a zone of lysed hyphae.

Many of the interactions described above were between species isolated from different resource units. However a few species were found together in the same log (often the same cross-section) and in the wood these species usually occupied discrete decay columns, the boundaries of which were marked by distinct black interaction zone lines. For example H. multiforme and H. nummularium were

isolated from separate decay columns in three consecutive cross-sections (B, C and D) of log HM2 (Figure 5.7*) and from two sections, C and F, of log S (Figure 5.6*). Similarly H. fuscum and H. nummularium occupied discrete decay columns in Section A, log VB (Figure 5.5*). Although some replacement reactions had been recorded between these species in culture (see above), there appeared to be no indication, such as diffuse, grey or broken "relic" zone lines separating different decay columns, that replacement had occurred in the wood. Occasionally different species were found together occupying the same decay zone. For example H. rubiginosum and H. nummularium in Section C, log RF (Figure 5.2*) and H. fuscum and H. nummularium in Section C, log F4 (Figure 5.4*) and in sections C and F, log VB (Figure 5.5*). A remarkable result was the isolation of "H. purpureum", H. multiforme and H. nummularium from a single small decay region in Section D, log S (Figure 5.6*). As "H. purpureum" and H. multiforme also occurred in adjacent decay columns, it may be that this small domain was originally that of H. nummularium and had subsequently been invaded by the other two species.

ii. Interactions between xylariaceous species and Hymenochaete corrugata.

Hymenochaete corrugata, a wood-decaying basidiomycete, was isolated from four hazel poles, originating from separate coppice stools in Friary Woods, Avon. The poles were also colonized by

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* These figures are in Chapter 5.

Hypoxylon fuscum (all four) and Hypoxylon multiforme (one). Experimental pairings between H. corrugata and these species resulted in deadlock and unilateral replacement reactions (Table 6.3). They also yielded some interesting interaction responses, as H. corrugata isolates exhibited two distinctive mycelial types when grown on 2% MA. These were an appressed yellow-brown pigmented ("flat") morphology type and a white aerial ("fluffy") morphology type. Where these two occurred adjacent to one another, a chestnut brown pseudosclerotial plate (PSP) was formed between them (Sharland, Burton and Rayner, 1986).

With H. fuscum, the H. corrugata mycelium at the interaction interface was always of the flat morphology type. Even if the initial inoculum was fluffy, it formed a PSP and became flat adjacent to the zone of contact. Most interactions (90%) between these two species (including all involving both H. corrugata mycelial types, as just described) resulted in deadlock (Figure 6.3 A). One genotype of H. corrugata in the flat mode was however, replaced by two H. fuscum genotypes (Figure 6.3 B) over a small distance ($\leq$ 5 mm) by white mycelial fans. These were composed of dense thread-like aggregations of hyphae. Conversely, mycelium of this same H. corrugata strain partially overgrew another H. fuscum genotype which was displaying restricted mycelial growth (i.e. slow radial extension rate, dense mycelial network, intense pigmentation - see Chapter 3, Section 3.3, i).

Deadlock was also the outcome in the majority (64%) of interactions between H. multiforme and H. corrugata, but in these reactions the mycelium of the basidiomycete remained committed to the morphological type of its inoculum. Even at the interaction interface the fluffy morphological type did not become flat, although a PSP was formed at this point in all pair combinations (Figure 6.3 C, D). Where unilateral replacement occurred it was always the fluffy mycelial type of H. corrugata that encroached upon H. multiforme, and a PSP was produced (by H. corrugata) at the limit of the replacement front.

In the wood H. corrugata and H. fuscum or H. multiforme were commonly isolated from mutually exclusive decay columns demarcated by zone lines (see Chapter 5, Figure 5.8). These were well-defined, distinct black lines and there was no visible evidence (such as diffuse "relic" zones lines separating decay columns), that replacement of one species by another had occurred. Hypoxylon fuscum and H. corrugata were however sometimes isolated from the same decay column, there being no apparent demarcation between them.

6.4 Discussion

This limited survey of interspecific interactions involving xylariaceous species demonstrated that these fungi are combative, the usual outcome of direct physiological challenge between different species being an inter-mycelial reaction resulting in deadlock or partial replacement. That they possess combative

ability is perhaps not surprising considering their apparent colonization strategy of latent invasion (discussed in Chapter 5, Section 5.4). According to Rayner and Boddy (in press) fungi which are to colonize a resource have two basic options. They can either establish in a formerly vacant domain via primary resource capture, or they can establish in a domain already occupied by another thallus by replacing it by secondary resource capture. Fungi that opt for the first alternative may do so by being stress tolerant, and as long as the stressful selective conditions to which they are adapted are maintained, colonization can proceed without the requirement of combative ability. However, if these stressful selective conditions are alleviated (the situation that applies to latent invasion) pressures increase either for exit, usually involving rapid commitment to reproduction, or for defence of captured domain. The xylariaceous species of this study seem to fall into the latter category. By contrast fungi that colonize via secondary resource capture require the capacity to take over successfully domain of another fungus, not merely to defend their own domain.

A combative hierarchy was evident, each xylariaceous species possessing a different combative ability. Daldinia concentrica appeared to be the most combative fungus as it replaced all other species with which it was paired. Hypoxylon multiforme and H. fuscum were also highly combative, replacing some species ("H. purpureum" and H. nummularium, and H. nummularium respectively) and resulting in deadlock with others. Hypoxylon fragiforme and "H. purpureum" were defensive, maintaining their own domains, but

failing to invade that of others. The weakest combatant was H. nummularium which was frequently replaced by a variety of species (H. multiforme, H. rubiginosum, D. concentrica and H. fuscum), but it was able to retain its domain with other beech (Fagus sylvatica) inhabiting Xylariaceae, "H. purpureum" and H. fragiforme.

A combative hierarchy such as this has been established for many of the fungi found most frequently in a study of felled beech (F. sylvatica) logs (L. Boddy, pers. comm.). When paired on agar Hypholoma fasciculare, Lenzites betulina, Psathyrella hydrophila and Sistotrema brinkmanii were most combative; Phallus impudicus, Phanerochaete velutina and Tricholomopsis platyphylla were slightly less combative; Coriolus versicolor and Stereum hirsutum weaker combatants and Armillaria bulbosa, Lopadostroma turgidum and Xylaria hypoxylon were least combative, being replaced by most other species.

The combative fungal interactions of these Xylariaceae appeared to be mediated primarily through inter-mycelial contact. Hence replacement was most frequently achieved by gross contact between mycelia, in which the thallus of one species seemed to grow over that of the other. This seemed to involve an alteration in morphology of the advancing mycelial front - a feature which will be discussed in more detail below. Similarly deadlock sometimes involved inhibition or suppression of extension by one or both colonies and production of pigment and lysis.

The inhibition of colony margin extension of H. fragiforme in pairings with "H. purpureum" may have been connected with the

strong bitter-sweet smell, reminiscent of bitter almonds, associated with the dull green (DG) mycelium of the latter. Volatile substances diffusing through common air space are known to elicit such inhibitory effects (Mowe, King and Senn, 1983). It is not clear what could have caused the suppression of growth in H. nummularium colonies paired with H. rubiginosum. Perhaps H. rubiginosum generated a pH that was unfavourable for H. nummularium mycelial extension, or produced an antibiotic that diffused freely through the growth medium. The restriction of colony margin extension in both species combinations seemed to involve changes in developmental regulation of the suppressed colony, as a result of interspecific confrontation (discussed below). Irrespective of the precise mechanism by which "H. purpureum" and H. rubiginosum brought about suppression of colony margin extension, the process in each case appears to be specific for H. fragiforme and H. nummularium respectively, as other species were not affected. It should be pointed out that if diffusible substances are involved, as conditions in natural situations may not allow their accumulation, these sort of interactions may not be significant in nature (Rayner and Boddy, in press).

In analysis of the role that species may play in the structure and development of natural fungal communities based on observations of interspecific interactions, it is important to bear in mind that the outcome of such interactions may be modified by any one, or any combination of an array of factors. These may include developmental status of the mycelia involved and abiotic variables, such

as gaseous regime an water potential, which may alter under different natural conditions. A possible example of the former is that pairings between H. nummularium versus H. multiforme and versus H. rubiginosum yielded both deadlock and replacement reactions. This demonstrates that different genotypes of the same species may not be consistent in their interactive performance, possibly due to genetic differences and/or differences in their physiological states. Examples of abiotic variables affecting interaction outcome are discussed below.

The standing tree or detached wood are heterogeneous environments with respect to water potential and gaseous regimes (Rayner and Boddy, in press) and changes in these have been demonstrated to modify the outcome of interspecific interactions. For example in the study of certain fungi causing decay in beech (Fagus sylvatica) logs mentioned earlier, cord-forming Basidiomycotina were able to replace many of the other species under an atmospheric gaseous regime. However, the outcomes were sometimes reversed under increased carbon dioxide and reduced oxygen regimes, so that Coriolus versicolor replaced Phallus impudicus and Phanerochaete velutina, and Sistotrema brinkmannii replaced Tricholomopsis platyphylla (L. Boddy, pers. comm.).

In studies of Ascomycotina and Basidiomycotina in ash (Fraxinus excelsior) branches (Boddy, Gibbon and Grundy, 1985; Boddy, Bardsley and Gibbon, 1987), under atmospheric conditions Daldinia concentrica readily replaced H. rubiginosum (as occurred in the

present investigation), but was deadlocked or partially replaced by Coriolus versicolor. However D. concentrica and H. rubiginosum deadlocked, whilst D. concentrica replaced C. versicolor, under increased carbon dioxide and decreased oxygen tensions. This gaseous regime is equivalent to that likely to occur in the standing tree, and in this respect the outcome of such interactions may be significant. Daldinia concentrica is frequently associated with decay in standing trees and yet can persist in fallen timber; H. rubiginosum also occurs in standing trees, but is easily replaced in detached wood, and C. versicolor is usually abundant on the latter, but unusual in standing trees.

Alterations to water potential also modified the outcome of interactions between these ash-inhabiting fungi. However D. concentrica was able to replace most of the species with which it was paired at -1.3 MPa and -2.2 MPa. It is not unreasonable to suggest that the apparent highly combative behaviour recorded for D. concentrica in these studies and in the present investigation, may also occur under certain conditions in nature. This may explain why this species has persisted in ash (Fraxinus excelsior) trunks at Bathwick Woods, Avon, felled 20 years ago without being replaced by other fungi (A.D.M. Rayner, pers. comm.).

Where zone lines were visible in wood, demarcating longitudinally extensive decay columns occupied by different xylariaceous species, the appearance and form of these lines invariably supported the observations of interactions in culture.

That is, well-defined dark brown or black zone lines, occurring singly, or in pairs running parallel to one another, between particular species in wood, corresponded to deadlock reactions in culture, whilst diffuse grey lines were associated with replacement. From this information it is hard to determine whether such confrontation between mycelia of different species arose following simultaneous colonization, or whether one or other species was established in the substratum first. However, where a decay column occupied by one species was surrounded by a continuous zone line, demarcating it from adjacent decay columns occupied by other species, this may indicate that colonization has occurred on different occasions. For example in log S, Section D (see Chapter 5, Figure 5.6) where "H. purpureum" was surrounded by H. nummularium and H. multiforme. This suggestion was made for the same situation in ash (Fraxinus excelsior) logs colonized by D. concentrica (Boddy, Gibbon and Grundy, 1985).

Where different species were isolated from the same decay column as occurred for example with Hymenochaete corrugata and Hypoxylon fuscum, this may have arisen through both species colonizing via latent invasion (see Chapter 5, Section 5.4). The two species may have been so irretrievably intermixed that they were unable to form discrete decay columns. Where however these particular species and H. corrugata and Hypoxylon multiforme occupied mutually exclusive decay columns, this presumably corresponds to deadlock in culture (Sharland, Burton and Rayner, 1986). That 90% of pairings between H. corrugata and H. fuscum and

64% of those between H. corrugata and H. multiforme resulted in deadlock, supports the view discussed above that these xylariaceous species are highly combative.

The replacement of H. fuscum by H. corrugata appears to be exceptional, as it only occurred between one combination of genotypes involving an H. fuscum strain in a restricted (R) mycelial mode (see Chapter 3, Section 3.3, i) and replacement was very limited. Frequently the H. corrugata mycelium was in the appressed (flat) morphology type (mode) adjacent to the interaction interface with H. fuscum, even where it had initially grown from the inoculum in the aerial (fluffy) mode. This is a parallel with observations made of intraspecific pairings between different genotypes of H. corrugata. Here, in addition to producing a luteus pigmented rejection zone as a result of somatic incompatibility, the flat mode was often produced in response to the interaction (Sharland, Burton and Rayner, 1986). With H. multiforme however, there was no alteration to the flat mode along the interaction interface and replacement by H. corrugata was always by the fluffy type.

There is considerable evidence that recognition responses in interspecific interactions seem to involve changes in developmental regulation of the type already discussed in Chapter 3 (Rayner and Coates, 1987; Rayner, Boddy and Dowson, 1987; Rayner and Boddy, in press). For example many cord-forming Basidiomycotina produce cords in response to interactions (Rayner and Webber, 1984). In the

present investigation the alteration of mycelial mode of Hymenochaete corrugata from aerial (fluffy) to appressed (flat) at the interaction interface in pairings with H. fuscum is just one example of this. Further examples will be provided later.

Why confrontation with H. fuscum mycelium appeared to trigger a switch from one developmental pattern to another in H. corrugata and yet confrontation with H. multiforme mycelium did not, is not clear. A detailed examination of the interaction at the hyphal level (such as that described for intraspecific interactions of H. nummularium in Chapter 7) would perhaps reveal differences between the two situations. The H. corrugata hyphae may be damaged by those of H. fuscum.

Damage to the mycelium was one of several situations, including interspecific and intraspecific interactions and subculture, which appeared to induce the switch from one mode to another in H. corrugata, although sometimes it occurred spontaneously (Sharland, Burton and Rayner, 1986). Damage to the mycelium during interspecific interactions may also be associated with the compaction of hyphae into pseudosclerotial plates (PSPs) which were produced in deadlock and ultimately in replacement interactions once replacement was halted. Lopez-Real and Swift (1977) suggested that the initial stimulus for formation of the PSP in the basidiomycetes Stereum hirsutum and Armillaria mellea may be a traumatic factor liberated by damage to hyphae.

The present investigation provides evidence that xylariaceous fungi themselves may undergo changes in mycelial mode in response to contact by mycelia of other species. The production of white mycelial fans composed of cord-like hyphal aggregations of H. fuscum that extended for a short distance into the domain of H. corrugata (Figure 6.3 B) is an illustration of this. Further, an alternative method of replacement effected by the formation of an effuse front of white aerial mycelium lacking olivaceous flecks in D. concentrica, appears to be another example of a morphogenetic shift at the interaction interface. Boddy, Gibbon and Grundy (1985) also reported that D. concentrica changed to a "floccose aerial mode" in the vicinity of other fungi under adverse gaseous regimes.

There is a notable distinction between this silky textured aerial mycelium of D. concentrica in interspecific interactions and the woolly textured aerial mycelium (am) that was produced in the interaction zone in intraspecific pairings (see Chapter 4, Section 4.3, i, (b)). This may be an important distinguishing feature between intraspecific and interspecific interactions of the Xylariaceae. The production of a woolly textured am may indicate fertility and could perhaps be used as a taxonomic criterion to test for intersterility.

Other species for which switches between distinctive mycelial modes had been observed in unpaired mycelia, such as H. multiforme, "H. purpureum" and H. serpens (Chapter 3, Section 3.3, ii, (b), (c) and (d)) did not exhibit any such mode switches in interspecific

interactions. This indicates that for these species confrontation with mycelia of other fungi does not appear to act as a cue for such developmental alteration. It may be that in nature these particular mycelial modes are not concerned with combat with other organisms, but some other aspect of colonization, such as initial establishment or exploration. As mentioned earlier, the mycelium of H. fragiforme possibly underwent a mode switch from an unrestricted (U) to a restricted (R) mycelial type (see Chapter 3, Section 3.3, ii, (a)) in resonse to pairing with "H. purpureum". The proximity of the "H. purpureum" mycelium appeared to suppress the colony extension rate of H. fragiforme and induce it to become heavily pigmented - features of R mycelium.

The apparent combative nature of the xylariaceous species in this study is perhaps a characteristic feature of their ecological (life) strategy (discussed further in Chapter 8), complementing their ability to colonize rapidly by latent invasion. Presumably in nature, where they are subjected to a diverse array of fluctuating environmental conditions,their combative ability alters. Usually they may be able to retain their domain, however in certain circumstances it may be diminished, as they are replaced by more combatant fungi, that are perhaps better suited to the particular external conditions. Hence a complex web of actively interacting factors ultimately determines the outcome of mycelial confrontations and the structure and development of natural fungal communities.

Table 6.1. Characteristic features of deadlock interactions between different paired Hypoxylon species on 2% malt extract agar after 14 d incubated at 20°C in darkness.

Species paired	Number of interactions		Confrontation zone			Other notes
	tested	showing deadlock	width (mm)	Appearance		
				From above	From below	
H. nummularium x H. fragiforme	12	12	> 10	H. nummularium mycelium was appressed but intact throughout the interaction zone and the pigment below was visible.	Herbage green pigment bounded on H. fragiforme side by a narrow (2 mm) sienna line and sometimes on H. nummularium side by a broad (10 mm) brick band.	-
H. nummularium x "H. purpureum"	16	16	7-10	H. nummularium mycelium was appressed and composed of lysed hyphae throughout the interaction zone and pigment underneath was visible. Around the inoculum "H. purpureum" mycelium was dull green turning buff conidial but surrounding this, in contact with H. nummularium, it was white to buff downy to cottony. Droplets of colourless exudate were apparent where the 2 colonies met in 8 interactions.	Brick to cinnamon or sepia pigment underneath H. nummularium.	"H. purpureum" colony was always surrounded by H. nummularium due to linear extension rate differences.
H. nummularium x H. multiforme	16	7	10	H. nummularium mycelium was appressed so that pigment underneath was visible. Adjacent to this was a narrow (1 mm) line of amber or isabelline H. multiforme mycelium.	Olivaceous black or sepia pigment in a diffuse band marked the underside of H. nummularium mycelium.	-
H. nummularium x H. rubiginosum	14	12	9-10	Mycelium of H. nummularium was appressed so that pigment below showed through.	Sepia and herbage green pigment in a diffuse band underneath H. nummularium was bounded by narrow (1 mm) chestnut line where it came into contact with H. rubiginosum.	H. rubiginosum colony was smaller than H. nummularium but H. rubiginosum seemed to be capable of limiting H. nummularium growth on uncolonized agar.

Table 6.1. (continued).

Species paired	Number of interactions			Confrontation zone		Other
	tested	showing deadlock	width (mm)	Appearance From above	From below	notes
H. fragiforme x "H. purpureum"	12	12	< 5	H. fragiforme mycelium was white and woolly textured but in area adjacent to interaction zone it was downy so that the pigment below was visible.	Coral or coral to rust pigment was produced by H. fragiforme colony particularly in the region of contact.	In all interactions linear extension rate of H. fragiforme colony appeared to be suppressed.
H. multiforme x "H. purpureum"	9	5	5	H. multiforme mycelium became flattened and appressed (hyphal network intact but sparse) where it came into contact with the "H. purpureum" colony.	Pigment was absent from this zone.	-
H. multiforme x H. fuscum	12	12	5	Mycelia of both species were appressed so that pigment below was visible. H. fuscum mycelium along contact zone was often marked by cinnamon flecks.	Dark herbage green or dull green pigment seemed to be produced by both species.	H. fuscum colony was always surrounded by that of H. multiforme due to differences in linear extension rates (as shown by comparison with control i.e.unpaired colonies).

Table 6.2. Characteristic features of unilateral replacement interactions between different paired xylariaceous species on 2% malt extract agar after 14 d incubation at 20°C in darkness.

Species paired	Number of interactions tested	showing replacement	'Dominant' or invading species	Replacement zone			Other notes
				Extent of replacement	Appearance From above	From below	
Daldinia concentrica (Dc) x *Hypoxylon fragiforme* (Hf)	10	10	Dc	Whole Hf colony was smothered by Dc mycelium.	White silky textured aerial mycelium of Dc in which olivaceous flecks (typical of Dc mycelium) were absent, replaced Hf.	Dark herbage green or more usually sepia pigment marked the underside of the invaded Hf mycelium.	Hf colonies barely extended beyond a few mm from the inoculum and were completely surrounded by Dc mycelium yet unpaired Hf colonies did not have restricted extension rates.
D. concentrica (Dc) x *Hypoxylon rubiginosum* (Hr)	19	19	Dc	Half (approximately 10 mm) the diameter of Hr colony (approximately 20 mm).	Dc mycelium (described above) overgrew Hr mycelium at the margins of its colony and often this zone was marked by droplets of brown or colourless exudate.	Sepia pigment at centre of Hr colony was surrounded by a narrow (1-3 mm) sienna band adjacent to a diffuse (< 5 mm) amber underneath the Dc mycelium.	-
D. concentrica (Dc) x *Hypoxylon nummularium* (Hn)	10	10	Dc	Whole Hn colony entirely (7/10 interactions) or nearly (except 2 mm band along one side of Petri dish - 3/10 interactions) smothered by waves of Dc mycelium.	Dc mycelium (described above) overgrew that of Hn.	Smothered Hn colony was sometimes underlain by amber pigment but more usually it was cinnamon (typical of un paired Hn colony) with a diffuse amber band (18 mm) adjacent to Dc inoculum. A narrower (< 5 mm) band of isabelline pigment at the advancing front of Dc mycelium.	-

Table 6.2. (continued).

Hypoxylon species paired	Number of interactions tested	Number of interactions showing replacement	'Dominant' or invading species	Replacement zone: Extent of replacement	Replacement zone: Appearance from above	Replacement zone: Appearance from below	Other notes
H. nummularium (Hn) x H. fuscum (H fus)	57	57 (25 type a 32 type b)	H fus	(a) 5–10 mm band of Hn colony.	A 'clear' band of sparse lysed Hn hyphae preceded the advancing front of H fus mycelium.	Citrine green or citrine green to herbage green pigment was bounded on the H fus side by a narrow (2 mm) isabelline line and the Hn side by a broad (5–10 mm) diffuse band of sepia pigment.	Replacement was halted by the sepia band of Hn so that it was restricted to the lysed zone.
				(b) Varied between different combinations of genotypes but usually 5–10 mm band of Hn colony.	A raised zone of H fus mycelium (buff or honey, pellicular to velvety textured) grew over Hn colony.	Hn colony was pigmented cinnamon except for a broad (5–10 mm) diffuse band of sepia pigment at the furthest extent of H fus mycelium.	As in (a) the sepia band of Hn marked the line along which replacement was halted.
H. nummularium (Hn) x H. rubiginosum (Hr)	14	2	Hr	A maximum of 15 mm	Honey/white, woolly to felty textured Hr mycelium grew over that of Hn where they came into contact and dark herbage green band (< 5 mm) of Hn lay adjacent to the Hr mycelial front.	Hn was pigmented isabelline to sepia and citrine green to dark herbage green in a band (< 5 mm) adjacent to this.	-
H. nummularium (Hn) x H. multiforme (Hm)	16	9	Hm	⩽ 20 mm	A wave of white Hm mycelium extended over a zone (15 mm) of appressed dark herbage green Hn.	Hn colony was pigmented isabelline at the centre and citrine green to dark herbage green towards the colony margin. Beside this a diffuse band (15 mm) of sepia pigment marked the furthest extent of Hm mycelium.	-

Table 6.2. (continued).

Hypoxylon species paired	Number of interactions tested	showing replacement	'Dominant' or invading species	Replacement zone: Extent of replacement	Replacement zone: Appearance From above	Replacement zone: Appearance From below	Other notes
H. multiforme (Hm) x H. mammatum (H mam)	12	12	H mam	10–20 mm	A wave of dense white aerial mycelium of H mam smothered the underlying Hm mycelium and was marked at its advancing edge by a narrow (1 mm) amber line in the Hm mycelium.	Hm was pigmented sienna to to umber in a broad (10–20 mm) band. This was bounded on the H mam side by a narrow (1 mm) chestnut line. Elsewhere the Hm colony was amber.	-
H. multiforme (Hm) x "H. purpureum" (Hp)	9	4	Hm	⩽ 10 mm across diameter of Hp colony.	Band of appressed Hm mycelium advanced over the edge (marked by a 1–2 mm line of buff mycelium) of the Hp colony (which remained dull green i.e. a 'typical' Hp colony).	Pigment was absent except for a honey line (1–2 mm) corresponding to the margin of the Hp colony.	The Hp colony was always surrounded by Hm mycelium. This corresponded to differences in their linear extension rates.

Table 6.3. Interactions recorded between Hypoxylon species and Hymenochaete corrugata on 2% malt extract agar after 14 d incubation at 20°C in darkness.

Hypoxylon species paired with Hymenochaete corrugata	Mycelial type of H. corrugata	Interactions: Total number tested	Number recorded as deadlock	Number recorded as replacement	Deadlock: Description of confrontation zone	Unilateral Replacement: 'Dominant' or invading species	Extent of replacement	Description of replacement zone
H. fuscum	fluffy (fy)	25	25	0	Adjacent to the interaction interface the fy white H. corrugata mycelium reverted to the ft morphology and a chestnut/black pseudosclerotial plate (PSP) was produced at this point. Neither H. fuscum nor H. corrugata mycelia were able to make territorial gain.	NA	NA	NA
	flat (ft)	5	2	3	The H. corrugata mycelium remained in the ft morphology even in the zone of contact with H. fuscum where it had produced a PSP and neither species was able to enter space occupied by the other.	H. fuscum (2 interactions)	≤ 5 mm	Fans of white velvety textured H. fuscum mycelium partially replaced the ft mycelium of H. corrugata.
						H. corrugata (1 interaction)	3 mm	The H. fuscum colony was intensely pigmented (dull green) and had a slow extension rate (see Chapter 3) and was surrounded and partially overgrown by H. corrugata.

Table 6.3. (continued).

Hypoxylon species paired with Hymenochaete corrugata	Mycelial type of H. corrugata	Interactions						
		Total number tested	Number recorded as deadlock	Number recorded as replacement	Deadlock Description of confrontation zone	Unilateral Replacement 'Dominant' or invading species	Extent of replacement	Description of replacement zone
H. multiforme	fluffy (fy)	10	6	4	A chestnut/black PSP was produced by H. corrugata colony where it came into contact with H. multiforme and the mycelia of neither species traversed this line. Ft morphology of H. corrugata was not evident in any interaction tested.	H. corrugata	10 mm	White fy aerial mycelium of H. corrugata extended over that of H. multiforme finally producing a PSP at the furthest extent of replacement.
	flat (ft)	1	1	0	The H. .multiforme colony was spatially dominant and surrounded the H. corrugata colony which was bounded by a PSP, but neither species was able to make a territorial gain.	NA	NA	NA

Figure 6.1. Deadlock interactions between Hypoxylon species after 14 d incubation at 20°C in darkness. In each combination of paired species both mycelia retain their original territories. Where the interaction zone is curved and one colony is surrounded by the other, this is due to differences in mycelial extension rates. (A,B) H. fragiforme (**Hf**) and H. nummularium (**Hn**). The interaction interface is marked by a band of appressed **Hn** mycelium (A - above) corresponding to a herbage green pigmented zone underneath (B) adjacent to which is a narrow line of sienna pigment produced by the **Hf** colony. (C,D) "H. purpureum" (**Hp**) and H. nummularium (**Hn**) (viewed from above and below respectively). As in (A) **Hn** hyphae are appressed and pigmented sepia in the region of contact with **Hp** mycelium, which itself has become buff and downy to cottony instead of dull green and velvety of "typical" **Hp** colonies.

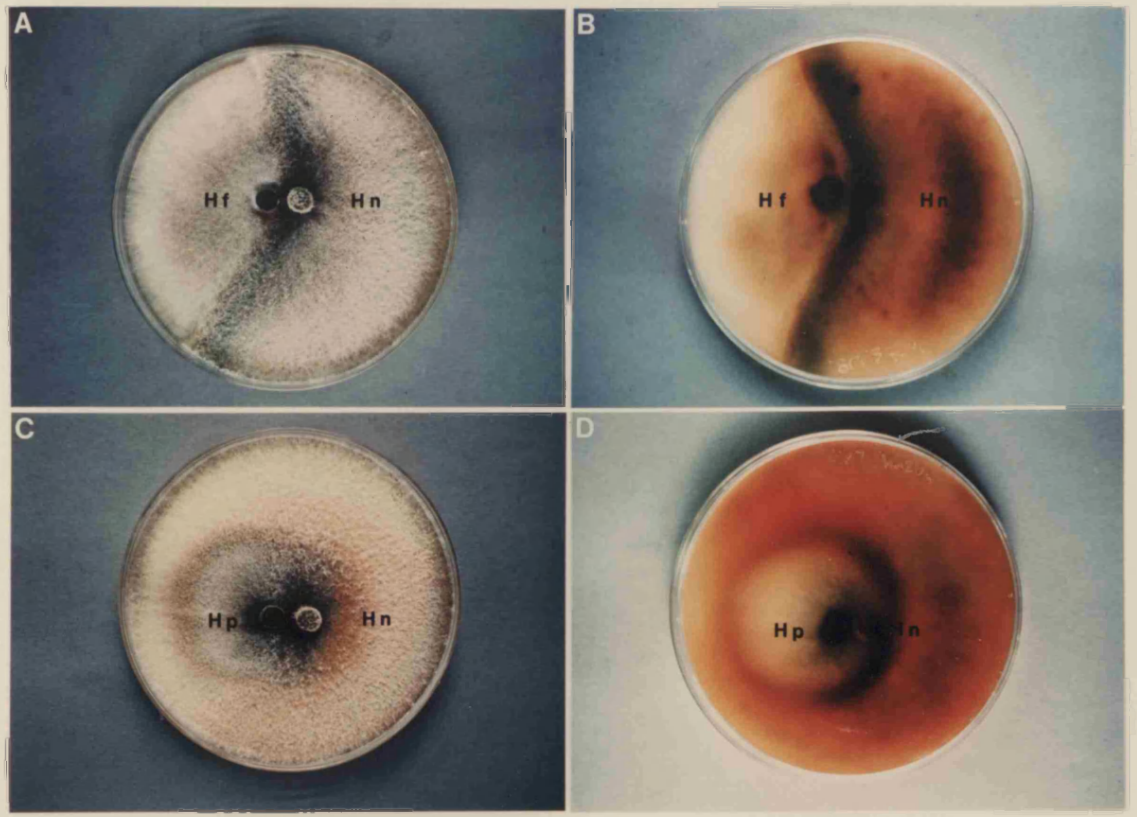

A
Hf
Hn
B
Hf
Hn
C
Hp
Hn
D
Hp

Figure 6.1. (continued).

Deadlock interactions between Hypoxylon species after 14 d incubation at 20°C in darkness. (E,F) H. rubiginosum (**Hr**) and H. nummularium (**Hn**). The extension rate of the **Hn** mycelium appears to have been suppressed (e.g. compared with that of **Hn** in (C) and (D)) by the proximity of **Hr** and particularly in the region of contact the **Hn** mycelium is appressed and intensely pigmented herbage green and sepia. The **Hr** colony seems to be unaffected and able to continue extending onto the uncolonized agar.(G,H) "H. purpureum" (**Hp**) and H. fragiforme (**Hf**). Extension of the **Hf** mycelium is inhibited (e.g. compared with **Hf** in (A) and (B)) and the colony is surrounded by strong coral to rust pigment; conversely extension of the **Hp** mycelium seems to be unaffected (compared to **Hp** in (B) and (C)) except that it is unable to grow towards the **Hf** colony.

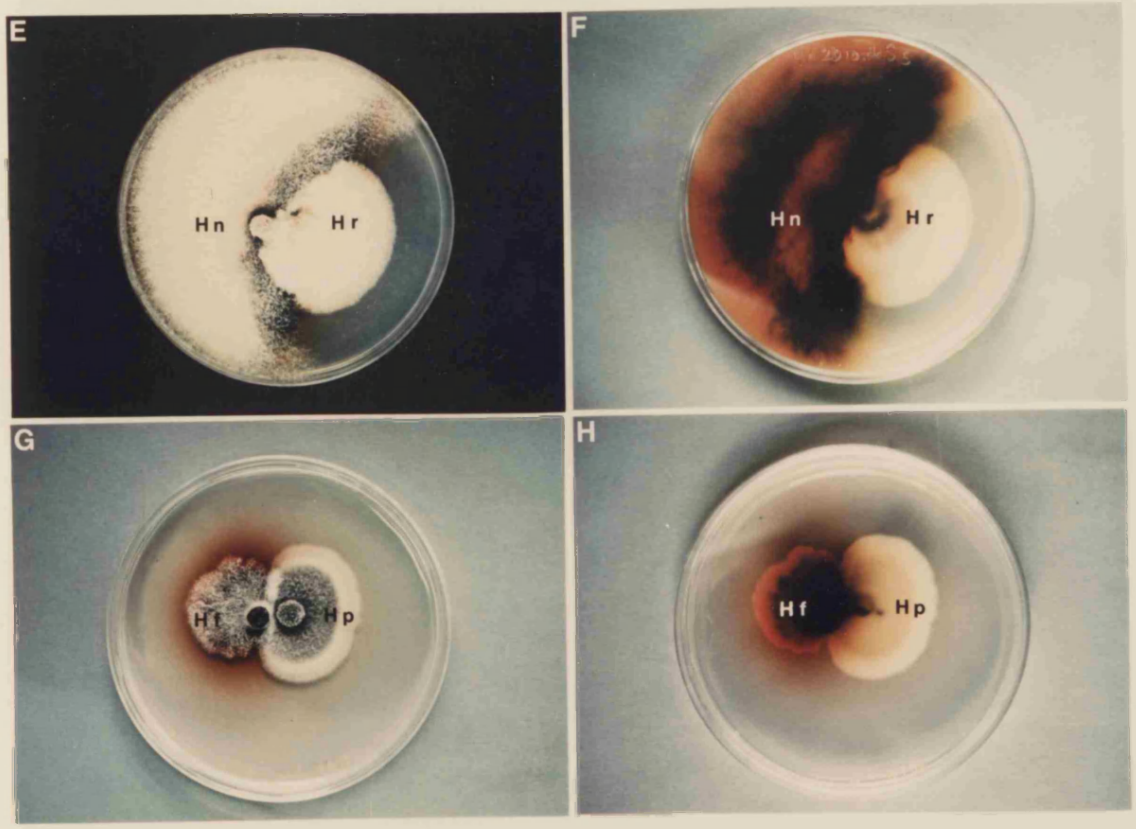

E
Hn
Hr
F
Hn
Hr
G
Hf
Hp
H
Hf
Hp

Figure 6.2. Unilateral replacement interactions between xylariaceous species after 14 d incubation. Replacement is mediated by the production of a wave of mycelium composed of densely packed hyphae which appear to "smother" the invaded colony. (A,B) Hypoxylon nummularium (**Hn**) and Daldinia concentrica (**Dc**) (above and below respectively). Note that the olivaceous flecks characteristic of **Dc** are absent from the silky textured mycelium that replaces almost the entire colony of **Hn** which is pigmented sepia. (C,D) H. nummularium (**Hn**) and Hypoxylon multiforme (**Hm**) (above and below respectively). Replacement of **Hn** by **Hm** is less extensive than in (A) and (B) and is halted along a broad sepia pigmented band in the **Hn** colony. (E,F) H. multiforme (**Hm**) and Hypoxylon mammatum (**H mam**). **Hm** mycelium is replaced by a mycelial wave of **H mam**, the advancing margin of which is indicated by a narrow amber line in the **Hm** mycelium (E, above). Underneath (F) this invaded zone is pigmented sepia. The mycelia of **Hn** and **Dc** in (A) both appear to be appressed. This is due to wetting from condensation which collected on the lid of the Petri dish and is not a result of the interaction.

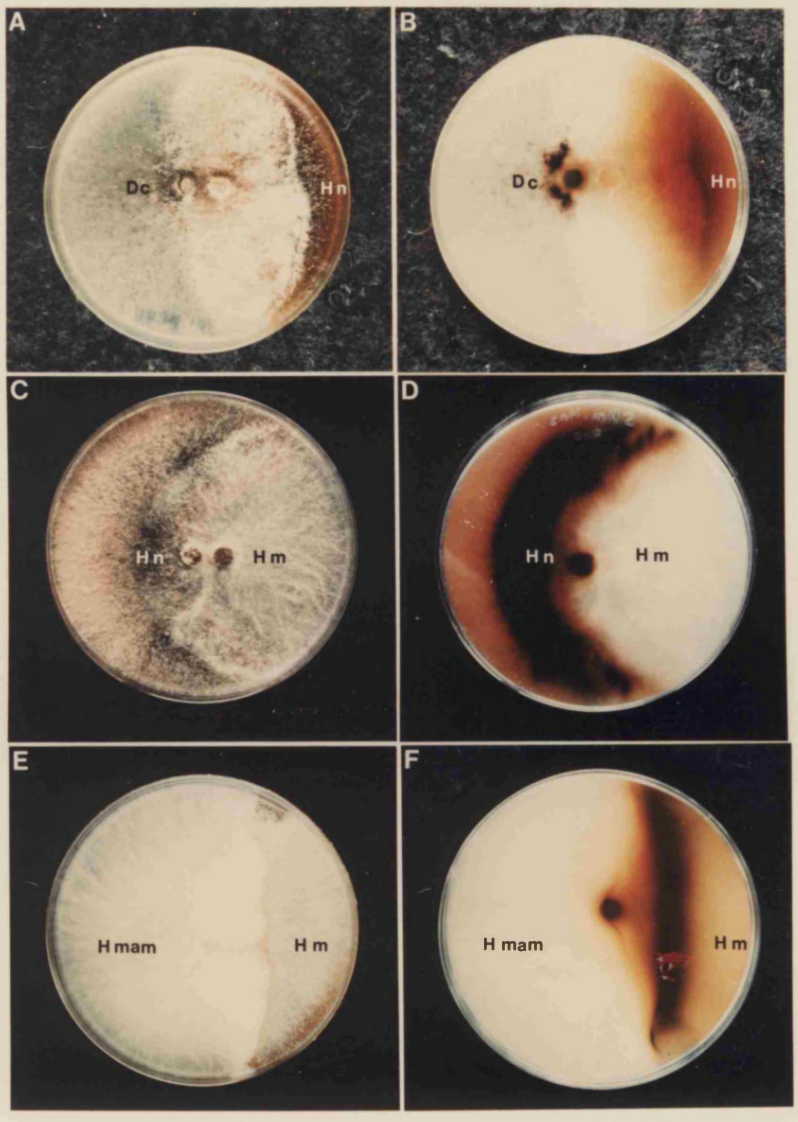
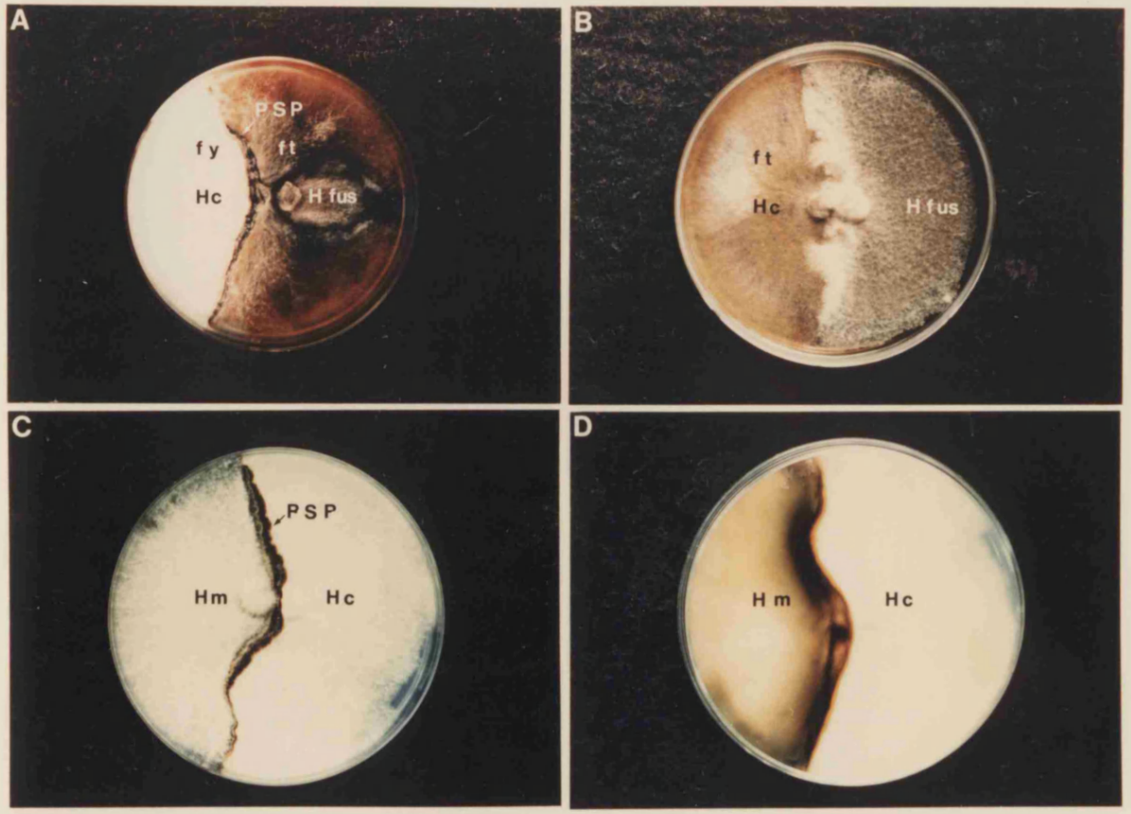

A
Dc
Hn
B
Dc
Hn
C
Hn
Hm
D
Hn
Hm
E
Hmam
Hm
F
Hmam
Hm

Figure 6.3. Interactions between Hymenochaete corrugata (**Hc**) and Hypoxylon species. (A) Deadlock with H. fuscum (**H fus**). Fluffy (**fy**) morphology of **Hc** forms a pseudosclerotial plate (**PSP**) and reverts to the flat (**ft**) morphology at the interaction interface with **H fus** (the small territory occupied by **H fus** compared with that of **Hc** is due to differences in mycelial extension rates). (B) Limited unilateral replacement by H. fuscum (**H fus**). Note the white mycelial fans of dense thread-like aggregations of **H fus** hyphae extending over the **Hc** (**ft** morphology type) mycelium. (C,D) Deadlock with H. multiforme (**Hm**). A PSP produced by the **Hc** mycelium (**fy** morphology type) marks the interaction interface with **Hm** and mycelia of neither species are able to traverse this line.


A
PSP
f y
ft
Hc
H fus
B
ft
Hc
H fus
C
PSP
Hm
Hc
D
H m
Hc

CHAPTER 7

CYTOLOGY OF HYPHAL INTERACTIONS

7.1 Introduction

Interactions that can occur within and between mycelia of higher fungi (such as those described in Chapters 4 and 6) represent the gross outcome of events within individual vegetative hyphae. Cytological studies of hyphal behaviour are therefore central to a complete understanding of the macroscopic interactions.

Fusions or anastomoses between hyphae occur readily in Ascomycotina and Basidiomycotina (Gregory, 1984; Aylmore and Todd, 1984a). They can occur both between hyphae of the same mycelium or genotype (self fusions), and between hyphae of genetically different mycelia (non-self fusions). Self fusions convert an otherwise radiating system of hyphae into a network, allowing conduction of food materials in diverse directions, recovery of the mycelium following injury enabling it to continue acting as a unit, and the binding of hyphae into plectenchymatous aggregations, such as fruit bodies. Non-self fusions allow the possibility of genetic exchange, the ultimate outcome of which in Basidiomycotina is determined by the relative expression of somatic incompatibility (rejection of non-self) and mating compatibility (override of non-self rejection) mechanisms (Rayner et al., 1984) (see Chapters 1 and 4, Sections 1.1, ii, (a) and 4.4 respectively). In many Ascomycotina, where mating is normally regarded as being confined

to specialized sexual organs, it may be reasonable to expect that where somatic non-self fusions occur between homokaryons, they would be followed by an incompatible reaction preventing the mixing of two different protoplasms. However, observations of intraspecific mycelial interactions in some xylariaceous species (e.g. Daldinia concentrica and Hypoxylon nummularium) have indicated that this may not be the case in all non-self fusions; an aerial mycelium (am) that is temporarily heterokaryotic is formed between some combinations of genotypes (e.g. those producing bow ties - see Chapter 4, Sections 4.3 - i, (b) and iii - and 4.4).

Some of the first observations of hyphal fusions, protoplasmic streaming and nuclear migration in the vegetative mycelium were reported by Buller (1931, 1933), but since this time there have been surprisingly few cytological studies. With regards to Ascomycotina these have included examination of "nuclear streaming" in Gelasinospora tetrasperma (Dowding, 1958) and the "lethal incompatibility reaction" in Neurospora crassa (Garnjobst and Wilson, 1956; Wilson, Garnjobst and Tatum, 1961). Nuclear streaming referred to the phenomenon of large numbers of nuclei rapidly migrating through the mycelium for long distances, passing from one compartment to the next via the septal pore. This only occurred under certain conditions, whilst under others the moving cytoplasm left the nuclei behind, fixed in the thin cytoplasmic lining of the cell wall. Light was suggested as a possible influence in the direction of streaming. The lethal incompatibility reaction in N. crassa followed hyphal fusions between strains with the same mating

type that failed to form heterokaryons.

All hyphal fusions were considered by Buller (1933) to be essentially tip-to-tip (i.e. end-to-end). However, a cytological study of self fusions between young hyphae of Penicillium claviforme showed that these hyphae were capable of tropic responses and formed tip (end)-to-side fusions. That is fusion of a hyphal tip with the lateral wall of another hypha (Watkinson, 1978). Tip-to-side fusions have since been observed in other species (Aylmore and Todd, 1984a; Todd and Aylmore, 1985; Ainsworth and Rayner, 1986).

Recently, light and electron microscope studies of hyphal interactions in wood-decaying Basidiomycotina have been reported, in which a technique was used that allows continuous observation of living material up to the point of fixation. The initial study, the cytology of hyphal fusion in Coriolus versicolor (Aylmore and Todd, 1984a) was followed by similar investigations in Schizophyllum commune (Todd and Aylmore, 1985) and Phanerochaete velutina (Ainsworth and Rayner, 1986; Aylmore and Todd, 1986a,b).

These studies reported on the sequence of events prior to, during and following self and non-self fusions. The pattern of septation and nuclear behaviour in self fusions of P. velutina differed from that in C. versicolor and S. commune, and it was suggested that this was probably because of differences in the control of numbers of nuclei within individual hyphal compartments.

Phanerochaete velutina is holocoenocytic (multinucleate hyphal compartments and "whorled" clamp connections occur in both homokaryons and heterokaryons). By contrast the other two species have homokaryons with uninucleate compartments (i.e. monokaryons) and mating-type heterokaryons with binucleate compartments (dikaryons).

In P. velutina septation was always directly across the fusion pore where numerous nuclei divided, rather than at the site of division of an individual nucleus or pair of nuclei as in C. versicolor and S. commune. Further, the nuclear replacement reaction of these species was not observed. This reaction involves migration of a donor nucleus, or pair of conjugate nuclei, via the fusion bridge towards a recipient hypha, in which the resident nuclei round up and degenerate. Division of the donor nuclei and associated septum formation then re-establishes the uninucleate or binucleate condition. Fusions between different heterokaryons in P. velutina resulted in a lytic reaction (i.e. rapid cytoplasmic lysis and vacuolation). Here the fusion pore never expanded fully and nuclei were rarely exchanged. By contrast in C. versicolor and S. commune fusions between dikaryons followed the same sequence of events as self fusions. It was suggested that rejection responses were slow to develop and/or were prevented by the nuclear replacement reaction. Lysis also occurred between sexually compatible homokaryons of P. velutina - dolipore dissolution and nuclear migration being observed, only occasionally.

The technique used in the light (phase-contrast) microscope studies of C. versicolor, S. commune and P. velutina outlined above was adopted here to examine the cytology of interactions, between living hyphae, within and between strains of Hypoxylon nummularium. This species was selected as it produced a variety of aerial mycelium interactions (narrow line, wide band, bow-tie, hour-glass and pincer - see Chapter 4, Section 4.3, i, (b)) and particular combinations of strains usually produced consistent interaction types.

7.2 Materials and Methods

i. Strains and culture procedures

Cultures of homokaryotic strains of Hypoxylon nummularium derived from single ascospores from the same perithecium, were maintained on 2% MA at 20°C in darkness as described in Chapter 2. Before the cytological studies these mycelia were grown on autoclaved (in distilled water at 115°C for 20 min) cellophane (350 P00 British Cellophane Ltd.) overlying 0.02% w/v malt extract agar (MA) (0.2 g Munton and Fison spray malt A; 20 g lab M agar No. 2 per litre). The resulting relatively thin monolayer of hyphae allowed easy observation.

ii. Preparation for microscopy

A microculture chamber was prepared according to the methods described by Aylmore and Todd (1984b). These are briefly outlined below. Aseptic technique was used throughout.

Re-usable chambers (1 mm thick, 25 x 75 mm aluminium slides with a central 16 mm diameter hole, made into a cavity by a 22 x 57 mm coverslip glued to the lower surface,from which two, 4 mm wide, 0.4 mm deep, diagonally opposed air vents extended) were dry sterilized at 80°C for a minimum of 24 h. Coverslips and microscope slides were washed with Linkgleam (Link Chemicals Ltd., London), rinsed with distilled water, cleaned with 50% (v/v) glacial acetic acid and absolute ethanol and rinsed again prior to autoclaving (along with a 10 ml syringe, filter unit and vaseline) at 121°C for 15 min.

Two to three drops of molten 0.02% w/v MA (as above but lab M agar No. 2 was replaced by Purified agar, Oxoid Ltd., Code L28) were placed in the centre of the cavity using the syringe and filter unit containing 0.7 μm particle retention glass fibre paper (Whatman GF/F). Coverslips were placed on either side of the cavity to support a microscope slide which was laid over the agar to create a flat tablet, the thickness of which was determined by these coverslips. When the agar had set, the glass slide and coverslips were removed and a thin smear of vaseline was melted around the cavity on the upper surface of the aluminium slide.

A piece of cellophane (6-7 mm^2) was cut from the margin of a single colony (self fusion studies) or to include the edges of two adjacent colonies growing towards one another (non-self fusion studies). This was transferred to the centre of the agar tablet and a drop of sterile distilled water was added, before a coverslip was

pressed gently, but firmly, onto the vaseline. The chambers were incubated overnight at 20°C in darkness supported on glass rod triangles in Petri dishes.

iii. Microscopy

The chambers were transferred to a Wild M20 (Heerbrugg) microscope with camera attachment and observed at 22-25°C by phase-contrast optics over periods up to 72 h. Photomicrographs were taken on Kodak Technical Pan film 2415 (ESTAR-AH Base). A total of 20 self fusions and 25 non-self fusions between sib homokaryons were examined in detail.

7.3 Results

i. Fusion types and their distribution

Fusions were categorised into three types following Buller (1933). These were hypha to hypha, hypha to peg and peg to peg. In this terminology a "hypha" was an ordinary vegetative hypha of some length growing freely in the culture medium. A "peg" was a very short special fusion hypha which had never grown freely in the medium, but was produced in response to the presence of another hypha or peg with which it was destined to fuse. These fusion types were subdivided into those that occurred between two apices or hyphal tips (i.e. tip-to-tip fusions), and those between a tip and a lateral wall (i.e. tip-to-side fusions). Hypha to hypha fusions were frequently of the tip-to-side type (81%). This trend was reversed in hypha to peg and peg to peg fusions which were mainly tip-to-tip (71% and 80% respectively). It should be pointed out

that the percentages expressed here and below are approximate frequencies based on overall observations.

The most commonly recorded self fusion type was peg to peg (57%) often resulting in the formation of H bridges between main hyphae (Figure 7.1). Hypha to peg fusions were less frequent (35%) and hypha to hypha were relatively rare (8%). By contrast hypha to hypha and hypha to peg appeared to be the usual fusion types in non-self combinations (50% and 42% respectively) and peg to peg were only occasionally recorded (8%).

ii. Hyphal responses preceding fusion

Hyphae responded similarly preceding both self and non-self fusions. Tip-to-tip fusions invariably appeared to involve pre-contact stimuli. For example in hypha to peg and peg to peg types, one hypha acting at a distance, stimulated another to produce an opposing peg (i.e. "teleomorphosis") and subsequently the two apices grew towards one another (ie. "zygotropism"). The converse situation seemed to predominate in tip-to-side fusions, such that homing of an extending hyphal apex towards a specific site or sites in a lateral wall was rarely observed in self fusions and never in non-self fusions. The maximum distance over which such homing occurred was 100 μm. Instead, most tip-to-side fusions seemed to be the result of random apex-lateral wall contact with neither of the participating hyphae displaying any obvious pre-contact responses.

Only a very few such tip-to-side encounters resulted in successful fusion. Usually contact resulted in a period in which growth was halted and the apex flattened against the side wall. This was followed by resumption of growth and deflection of the apex over or along the lateral wall of the hypha.

Sometimes a tip of a hypha (A) would grow towards, and perpendicular to, the lateral wall of another hypha (B), but before making contact became re-orientated so that it grew parallel to B for a short distance (up to 50 μm). Growth of A was then halted and subsequently a peg would develop on A or B adjacent to, and directed towards, the other hypha which would respond by also producing a peg. Eventually the pegs would make contact and fuse. This repulsion followed by peg to peg fusion was a relatively common phenomenon in both self and non-self combinations. Occasionally the process would be halted at contact prior to fusion (3% in self and 12% in non-self).

Generally fusions were infrequent between very young hyphae, so that they came into contact and grew over or along one another. Similarly the older compartments of well-vacuolated main hyphae appeared to be unreceptive to fusions. It seemed that more encounters resulted in fusion within a strain than between strains. Hence in non-self combinations overgrowing hyphae or contacts without fusion occurred six times more frequently than fusions. By contrast in self combinations overgrowing hyphae or contacts occurred two times more frequently.

Fusions involving pegs were frequently directed towards septal regions. This was particularly noticeable in peg to peg fusions (72% associated with septa). Usually such fusions occurred towards one or other side of the septum and never occurred on both sides. Sometimes where two hyphae lay parallel to one another a peg produced on one hypha seemed to induce two pegs to form opposite on the other hypha. The three pegs grew towards one another and came into contact but only one peg of the pair fused with the"initiator" peg. Growth of the "unsuccessful" peg then usually became redirected away from the fusion (Figure 7.2).

iii. Events at and after self fusion

The sequence of events preceding, at and after a self fusion are shown in Figure 7.3. This type of fusion will be referred to as an open pore/cytoplasmic exchange (OPCE) fusion and is described below.

After contact and prior to the opening of the fusion pore there was a period (26-61 min) in which the interface between the two surfaces expanded, becoming flattened as the hyphae appeared to be pressed together. In one self fusion (hypha to hypha, tip-to-tip) this period was divided into an initial phase (8 min) of flattening, an intermediate phase (6 min) in which one hyphal tip became swollen, and a final phase (17 min) when no morphological changes occurred. Towards the end of the contact pre-fusion period sometimes one or more nuclei (hyphal compartments were multinucleate) aggregated in the tip (tip-to-side fusions), or in

one of the tips (tip-to-tip fusions), and one or more nucleolus exhibited periods of intense Brownian motion.

The fusion pore formed in the centre of the contact zone sometimes enlarging (over a period of 5 min) until it occupied almost the whole region. Once the pore was open cytoplasmic granules and vacuoles moved into the recipient hypha, vacuoles becoming constricted as they passed through the pore. The movement of cytoplasm into the recipient hypha was particularly rapid in one fusion. Here, mitochondria, apparently attached to the compartment wall, oscillated in the stream of cytoplasm which flowed through three adjacent compartments, and continued to do so (though more slowly as time proceeded) up to 2 h following fusion.

At fusion the nuclei in the tip were no longer visible. Later (at least 1 h after fusion) they appeared again in both fusion compartments, and movement of cytoplasm occurred in both directions through the pore. Once (a hypha to peg fusion) a change in direction of cytoplasmic movement through the pore was observed only 9 min after fusion. This seemed to be the result of another fusion nearby.

Hypha to hypha fusions remained open for at least 48 h. However, in fusions involving pegs, a septum was usually laid down across the pore between 3 and 20 h after fusion. Alternatively the pore remained open, but a septum was formed at the base of a peg (or sometimes at the base of both pegs in a peg to peg fusion)

between 6 and 15 h following fusion.

iv. Events at and after non-self fusion

These depended on the macroscopic mycelial interaction type produced in culture on 2% MA between the particular strains that were paired. Strains that resulted in weak narrow line reactions (i.e. a line of demarcation was discernible between the colonies as they were morphologically distinct, but neither aerial mycelium (am) nor pigment were present) followed the same sequence of events as OPCE fusions. Strains that yielded pincer and bow tie reactions produced what will be referred to as a contact/lytic response (CLR) "fusion" (Figure 7.4). These are described below.

With respect to strain combinations that produced weak narrow line reactions contact was usually followed by a period (23-43 min) in which the contact area was increased prior to immediate opening of the fusion pore. However, on two occasions opening was delayed, occurring 1 h 50 min and 2 h 51 min respectively after contact. The fusion pore was usually centrally positioned, but occasionally it was to one side. Once it opened fully and it occupied the full width of the contact interface.

As in self OPCE fusions nuclei were not usually visible at the time of fusion, but between 39 min and 1 h 25 min afterwards, two or three nuclei often passed through the fusion pore. However, on one occasion a nucleus was observed to traverse the pore 7 min after fusion and move through six adjacent hyphal compartments. It

was propelled by a stream of cytoplasm, possibly created by the release of pressure built up prior to fusion. Movement was slowed in the fifth and sixth compartments (the nucleus came to rest here) and ceased 24 min after fusion. There was no evidence of septal erosion - the nucleus seeming to cross intact septa.

Numerous small (< half the width of a hypha) spherical vacuoles often formed in the recipient compartment after fusion and these increased in number over 12 h. Fusions rarely remained open, usually the pore was closed by a septum 15 to 20 h following fusion and once it closed 1 h 31 min afterwards.

In pincer and bow-tie reactions a refractile band became apparent (2 h 40 min to 4 h after hyphal contact) at the interface between the two surfaces. Alternatively whilst the interface apparently remained intact, a process of vacuolation was initiated inside one or both participating hyphal compartments. These vacuoles, in contrast to those described above, were frequently rectangular and extended across the width of the hypha. A fusion pore like those observed in OPCE fusions was not visible in either of these situations, although in the former it may have been present, but obscured by the refractile band. Sometimes this refractility expanded unidirectionally, an entire hyphal tip becoming refractile.

Later (4 h 12 min - 12 h after contact) there was progressive, usually unilateral (see below) vacuolation and accompanying this

the cytoplasm became increasingly granular. Often as vacuolation proceeded there was an increase in refractility. Eventually (14 - 24 h following contact) the fusion compartment(s) was left as a hyphal ghost, empty of cytoplasm.

The extent of the lytic reaction in CLR "fusions" was usually restricted to either one (unilateral) or both (bilateral) participating compartments. Unilateral lysis was always a feature of interactions between genotypes that yielded pincer type reactions and it was always the "suppressed" genotype (see Chapter 4, Section 4.3, i, (b)) that was lysed. Genotypes that produced bow-tie interactions also often exhibited unilateral lysis (Figure 7.4) (and frequently the same genotype was lysed). In both cases regeneration of new hyphae behind the lysed compartments was common. These young hyphae grew over and/or around the affected area and took part in normal self OPCE fusions or made non-self contacts. It was intriguing that following the latter, the lytic reaction was not repeated, at least during the observation period (often restricted by now as visibility deteriorated with length of incubation in the microculture chamber). In one instance instead of a branch hypha developing behind the ghosted compartment an intra-hyphal hypha was formed. Its growth however was halted before it encountered the hypha of the other strain.

There were some notable exceptions to the above generalization that weak narrow line reactions involved OPCE fusions, whilst CLR "fusions" were associated with strains that produced pincer and

bow-tie reactions. A CLR "fusion" was recorded between one combination of strains giving a narrow line reaction, as well as the OPCE type fusion. However, in this case, the lytic reaction only resulted in the participating compartments becoming refractile and they were not left as ghosts. The converse situation occasionally occurred in strains that yielded pincer and bow-tie reactions. In two such non-self fusions (a hypha to hypha; pincer reaction and a hypha to peg; bow-tie reaction) an OPCE fusion response was observed. Contact was followed by 23 or 35 min respectively of expansion of the contact area, opening of the fusion pore and cytoplasmic exchange. In the former, 6 h 37 min after fusion, septa were laid down on either side of the pore, whilst in the latter the pore remained open throughout observation.

7.4 Discussion

This study of the cytology of hyphal interactions in Hypoxylon nummularium has revealed that hyphal compartments are multinucleate, and hyphal responses prior to fusion appear to be similar in self and non-self strain combinations. However, events at and after fusion may depend on recognition of self or non-self allowing varying degrees of acceptance and rejection (see Chapter 4, Section 4.4). Acceptance appears to be expressed where open pore/cytoplasmic exchange (OPCE) fusions occur, as these act as bridges enabling protoplasmic continuity between otherwise separate radiating hyphae. Rejection on the other hand, is perhaps manifested as contact/lytic response (CLR) "fusions" where the lytic reaction at the fusion site acts as a barrier preventing the mixing of protoplasms.

Hyphal behaviour prior to fusion appears to be consistent with observations recorded in previous cytological studies. For example the frequent occurrence of tip-to-side fusions was consistent with observations in Penicillium claviforme (Watkinson, 1978), Coriolus versicolor (Aylmore and Todd, 1984a), Schizophyllum commune (Todd and Aylmore, 1985) and Phanerochaete velutina (Ainsworth and Rayner, 1986). That in H. nummularium these usually seemed to result from random apex-lateral wall encounters, is similar to the situation recorded for C. versicolor and in contrast to that in P. velutina where a homing response was involved.

As mentioned earlier this tip-to-side fusion is at odds with Buller's (1933) proposal that hyphal fusions exclusively occurred between hyphal apices and invariably involved pre-contact stimuli. Nevertheless, this latter pattern of fusion was also recorded in the present study. Little is known as to the mechanism involved in such behaviour, although a number of hypotheses have been suggested. These include a suggestion that a growing hyphal tip produces a diffusible chemical that initiates teleomorphosis and zygotropism (Raper, 1952), and another that fusion between tips is the result of overlap of haloes of low concentration staling products (Park, 1961, 1963; Robinson and Park, 1965). However, neither hypothesis accounts for all observations, for example, the mutual repulsion between main hyphal tips mentioned by Burnett (1976) and seen in P. velutina (Ainsworth and Rayner, 1986). The

long range (up to 250 μm) curvature (homing) of hyphae towards specific sites in the lateral wall of recipient compartments observed in P. velutina, led to the proposal that this behaviour was a site-directed response, involving specific receptive sites.

These specific receptive sites may initiate the homing response. Whether the stimulus from this site is physical or chemical is unclear, but it was suggested that the Spitzenkörper of the homing hypha may play a role in detecting it (Ainsworth and Rayner, 1986). Ascomycotina, like Basidiomycotina possess a Spitzenkörper and in this respect it may be significant that a homing response was observed, albeit infrequently, in H. nummularium. However, the Spitzenkörper of Ascomycotina differs in structure from that of Basidiomycotina. In the former this dark apical body is composed of a dense clump of microvesicles surrounded by further macrovesicles and microvesicles, whilst in the latter it appears as a vesicle free region surrounded by macrovesicles and microvesicles. It seems possible therefore that Spitzenkörper may fulfil different functional roles in the two groups.

Distinctions between Ascomycotina and Basidiomycotina, such as the one mentioned above, should be borne in mind throughout this discussion where comparisons are made between the observations of the present investigation and those of cytological studies in Basidiomycotina. It is not clear for example how differences in

septal structure between the two groups affects the ease with which protoplasm may move between hyphal compartments. The elaborate and highly differentiated dolipore septa of Basidiomycotina presumably considerably restricts intercompartmental movement, so that septal erosion is required to allow large organelles, such as nuclei, to traverse them. By contrast the single central pore in the simple diaphragm septa of many Ascomycotina perhaps allows a relatively unimpeded passage of protoplasm between compartments.

Further, in the event of injury to the mycelium the mechanisms by which septa can be plugged differs between Ascomycotina and Basidiomycotina. Septal sealing in Ascomycotina may involve occlusion by round or oval shaped membrane-bound Woronin bodies or deposition of electron-dense material in the region of the pore. A study of septal sealing following damage in the basidiomycete Coriolus versicolor showed that there was instantaneous plugging of the pore channel by electron-dense material. This was followed by detachment of the septal apparatus in the ruptured compartment and re-modelling of the septal swelling on the other side of the wall, to give a permanent seal (Aylmore, Wakeley and Todd, 1984). The relative speed with which septal sealing can occur in Ascomycotina and Basidiomycotina may be significant with respect to the extent, in terms of the number of hyphal compartments involved, of a lytic reaction (see below).

With regards to hyphal responses preceding fusion, there are certain parallels between those observed here in H. nummularium and

those recorded for P. velutina (Ainsworth and Rayner, 1986). For example fusions in both species (particularly those involving pegs in H. nummularium) were invariably associated with septal regions. Further, that fusions in H. nummularium occurred well behind young tips, but not in old "mature" hyphae, is consistent with observations in P. velutina. These points in this basidiomycete were taken to suggest that the recipient sites were incipient branch sites of limited duration.

That peg to peg fusions were the most frequently recorded self fusion type in H. nummularium whilst hypha to hypha and hypha to peg were the most commonly recorded non-self fusion types, may merely be a result of hyphal alignment on the cellophane. As self fusion studies employed a small area ($< 4\ mm^2$) of mycelium cut from the margin of one colony, the hyphae were invariably growing parallel to one another. By contrast, non-self fusion studies included mycelia of two adjacent colonies growing towards one another. Hence their hyphae were likely to interdigitate and perhaps made more encounters involving hyphae than pegs.

The sequence of events at self fusion of H. nummularium had some features in common and some in contrast with self fusions observed in other fungi. For example the expansion of the interface between two participating hyphae and/or pegs following contact and prior to opening of a fusion pore was reported in P. velutina (Ainsworth and Rayner, 1986) and C. versicolor (Aylmore and Todd, 1984a). However the period over which this occurred in

H. nummularium (26-61 min) was considerably longer than either of these other two species (5-20 min for P. velutina and 15-20 min for C. versicolor). Nevertheless, that fusion in H. nummularium involved the formation of a single opening or pore in the interface region, and that this expanded, sometimes until all evidence of a cross-wall disappeared, was consistent with self fusions in the Basidiomycotina.

Observations of cytoplasmic movement across the fusion pore in H. nummularium, including flow in both directions, was also consistent with those of P. velutina and C. versicolor (Ainsworth and Rayner, 1986; Aylmore and Todd, 1984a). Indeed, the bulk flow of cytoplasm through three adjacent compartments following fusion which was recorded only once in H. nummularium, was similarly a rare event in C. versicolor.

Comparisons of nuclear behaviour at self fusion between H. nummularium and the Basidiomycotina mentioned above cannot be made. This is because the sporadic observations of H. nummularium nuclei at and after fusion make it impossible even to speculate as to how they act. It is not clear for instance whether nuclei move across the fusion pore, and if they do, whether they replace the resident nuclei in the recipient hypha, as in C. versicolor and S. commune, or not as in P. velutina (Aylmore and Todd, 1984a; Todd and Aylmore, 1985; Ainsworth and Rayner, 1986). Cytological studies of hyphal interactions in other xylariaceous species may clarify this situation, as their nuclei may be more conspicuous than those of H. nummularium.

The observed septation in self fusions of H. nummularium may be associated with nuclear behaviour. In C. versicolor and S. commune septa were formed at the sites of division of individual nuclei or pairs of nuclei (Aylmore and Todd, 1984a; Todd and Aylmore, 1985). By contrast in P. velutina septa were laid down directly across the fusion pore where numerous nuclei divided (Ainsworth and Rayner, 1986). Septation in H. nummularium frequently occurred across the fusion pore, or at the base of one or both pegs in peg to peg fusions, whilst hypha to hypha fusions seemed to remain open.

There appeared to be no real difference between the open pore cytoplasmic exchange (OPCE) fusions that occurred in non-self combinations (between strains that in culture on 2% MA yielded weak narrow line reactions) and those that were recorded in self combinations. It may be reasonable to suggest therefore, that as on one occasion a nucleus was seen to traverse a fusion pore 7 min after fusion in the former, nuclei do move into recipient hyphal compartments following fusion in the latter. However, whether nuclei migrate beyond the recipient compartment, and whether nuclei merely pass through the central septal pore without septal erosion (required for nuclear migration in Basidiomycotina, see above) in self and non-self combinations, needs to be considered following further observations. The one instance when the nucleus, having crossed the pore, was seen to move through six adjacent compartments of the recipient hypha, apparently crossing intact septa, may not be representative of the normal course of events. It seems likely that it was an unusual event, resulting from the

release of cytoplasmic pressure that had built up in the "donor" mycelium. It should be pointed out, however, that such a "violent expulsion of protoplasm which carries the nucleus with it" has been implicated as a mechanism for transference of nuclei in hyphal fusions of Typhula trifolii (Noble, 1937).

In marked contrast to the OPCE fusions were the contact lytic response (CLR) "fusions". The vacuolation, refractility and eventual "ghosting" of hyphal compartments involved in CLR fusions were reminiscent of the lytic reaction reported between heterokaryons and some mating compatible homokaryons of P. velutina (Ainsworth and Rayner, 1986). However, the initial events and timing were different in H. nummularium. Unlike P. velutina there was no evidence (such as movement of granules into the recipient hypha) for the existence of a fusion pore, nor for the temporary cessation of cytoplasmic activity (manifested by the motion of organelles). Instead, the first visible event of lysis in H. nummularium was either the appearance of a refractile band in the region of contact, or the initiation of a vacuolation process inside one or both hyphal compartments.

With regards to timing of the lytic reaction, in P. velutina this depended on the strain combinations that were paired. Hence in fusions between heterokaryons there was rapid development of a unilateral or bilateral lytic reaction immediately following fusion. This was also the situation between some of the mating compatible homokaryons, whilst others resulted in delayed lysis.

Lysis in H. nummularium always took some time to develop, so that the first signs appeared 2 h 40 min to 4 h after contact, but it was not complete until 14 h to 24 h following contact.

Some features of lysis in H. nummularium are similar to those of the lethal incompatibility reaction described between certain strains of Neurospora crassa (Garnjobst and Wilson, 1956). These include progressive vacuolation and granulation of the cytoplasm in participating compartments. Other signs of incompatibility in N. crassa were not observed in H. nummularium. For example in N. crassa large biconvex plugs developed in the septal pores, sealing off the damaged cells from adjacent healthy ones. Also septa bowed inwards towards the fusion, apparently as a result of loss of turgor in fused cells.

A characteristic of H. nummularium CLR "fusions" that requires further attention is the apparent lack of formation of a fusion pore mentioned earlier. That is the lytic response seemed to arise following contact only. In this respect CLR "fusions" resemble the phenomenon of hyphal interference. Increased refractility and vacuolation for example occur when hyphae of Ascobolus crenulatus are contacted by those of Coprinus heptemerus (Ikediugwu and Webster, 1970; Ikediugwu, 1976). However, electron microscope studies of CLR "fusions" in H. nummularium are required to ascertain whether or not continuity occurs at any time between two participating hyphae and/or pegs. Further, such studies may elucidate other aspects of the lytic response, such as the

effect(s) on membrane systems and organelles (e.g. mitochondria).

The lack of repetition of a lytic response following contact between non-self hyphae that regenerated behind lysed compartments of CLR "fusions", may be significant in relation to macroscopic interaction patterns produced in culture on 2% MA plates. CLR "fusions" predominated between strains that yielded interactions marked by abundant aerial mycelium (pincer and bow-tie reactions). It may be that after the initial rejection, manifested as CLR "fusions", there is acceptance of non-self. In the absence of the coverslip directly above the hyphae in the microculture chamber, perhaps OPCE fusions or some other fusion type may occur as a result of these secondary non-self encounters. These may result in the development of the temporarily heterokaryotic aerial mycelium shown to exist in the interaction zone in Chapter 4 (Sections 4.3 - i, (b) and iii - and 4.4). If this hypothesis is correct, the sequence of initial rejection followed by acceptance between non-self, reflects the observations of macroscopic interaction patterns in culture of for example Hypoxylon fuscum. Different genotypes initially produced a trough of sparse mycelium along the interaction interface. In some strain combinations this non-self rejection was subsequently overridden and there was a degree of non-self acceptance manifested in the development of a lens shaped area of temporarily heterokaryotic mycelium (Chapter 4, Sections 4.3 - i, (b) and iii - and 4.4).

It is significant that interactions between genotypes that produced pincer reactions on 2% MA always resulted in CLR "fusions" marked by unilateral lysis of the "suppressed" genotype (Chapter 4, Section 4.3, i, (b)). Hence mycelium of the "unsuppressed" genotype seemed to remain undamaged following the encounter. This must be an advantage to this genotype with respect to its relative ability to become spatially dominant.

Strains yielding weak narrow line reactions in culture on 2% MA, that is reactions in which aerial mycelium was absent, mainly produced OPCE fusions, the fusion type observed in all self combinations. This indicates that these strains may be genetically very similar with respect to somatic incompatibility loci. However, the occasional occurrence of a CLR "fusion" shows that there is some rejection of non-self.

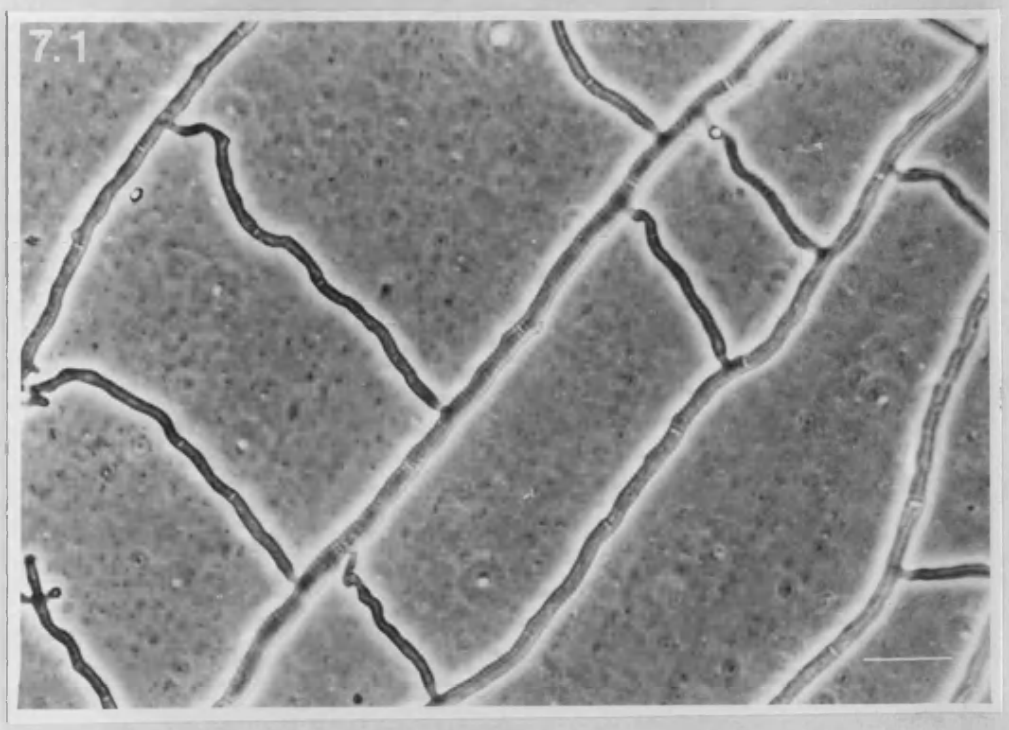
Based on the observations of the present investigation it seems reasonable to suggest that, as mentioned above, self acceptance is expressed as OPCE fusions allowing protoplasmic exchange, whilst rejection is manifested as CLR "fusions", preventing the mixing of protoplasm. Ultimately the interaction patterns observed in culture on 2% MA are the gross outcome of the combination of fusion events within individual vegetative hyphae. In this study two different fusion events were observed. OPCE fusions were associated with self fusions and sometimes non-self fusions whilst CLR "fusions" were only seen in non-self combinations. In aerial mycelium interactions the initial rejection, manifested as CLR "fusions", may be followed

by aerial mycelium development. As subsequent encounters between hyphae of different strains did not produce lysis, perhaps the rejection had been overridden. As explained earlier, had it not been prevented, an aerial mycelium may have developed through OPCE fusion or some other fusion type between the different strains.

To test the ideas outlined above, further careful observation of the cytology of hyphal interactions are required. In particular events after CLR "fusions" should be monitored in all interaction types marked by the production of aerial mycelium (i.e. strong narrow line, wide band and hour-glass as well as pincer and bow-tie reactions). If possible aerial mycelium should be allowed to develop at this stage and its genetic composition, with respect to somatic incompatibility, tested using the single hyphal tip method described in Chapter 2 (Section 2.6). Further, there is a need to extend cytological studies of hyphal behaviour between strains to include other xylariaceous species that yield aerial mycelium interactions and those that produce trough or barrage reactions.

Figure 7.1. H bridges between three main hyphae of a single mycelium (self fusions) resulting in a ladder-like appearance. Bar marker represents 10 µm.

Figure 7.2. Peg to peg self fusion. (A) Two pegs from one main hypha grow towards a third peg produced by another main hypha. Bar marker represents 10 µm.


7.1

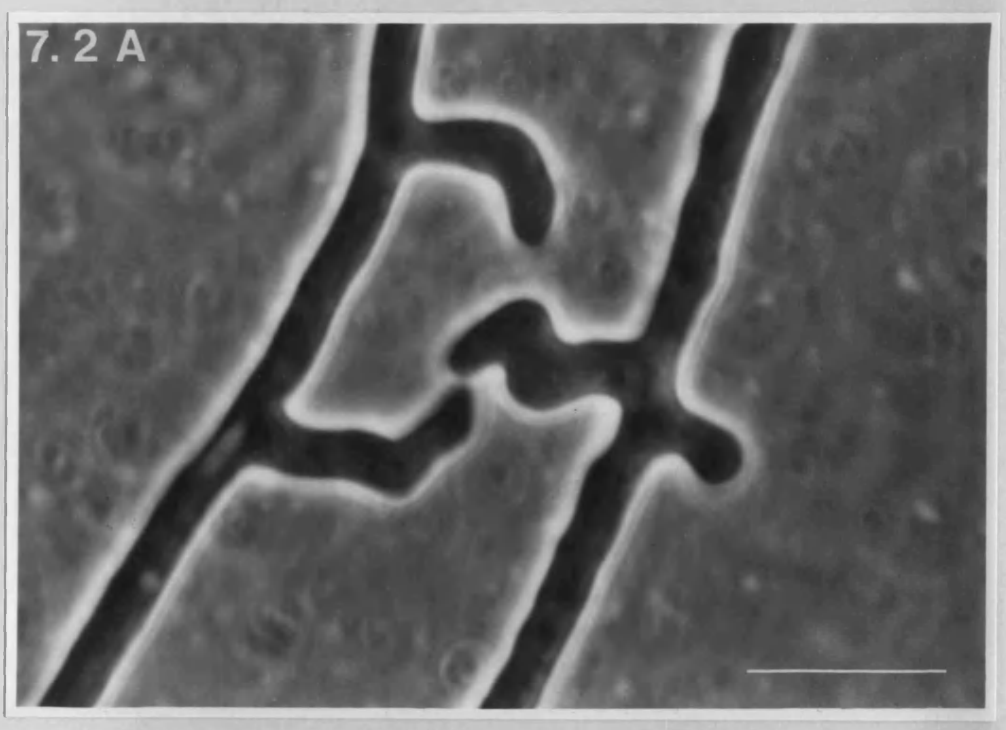
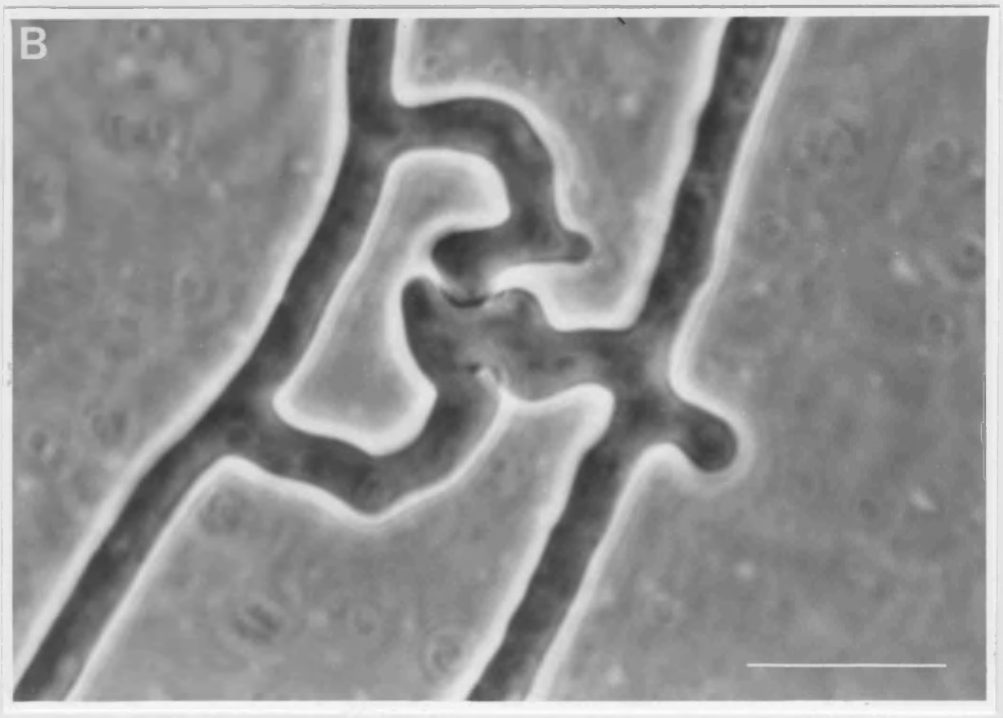

7. 2 A

Figure 7.2. (continued).

Peg to peg self fusion.

(B) All three pegs make contact, but only two fuse, growth of the "unsuccessful" peg being redirected (C) away from the fusion. Bar marker represents 10 μm.


B

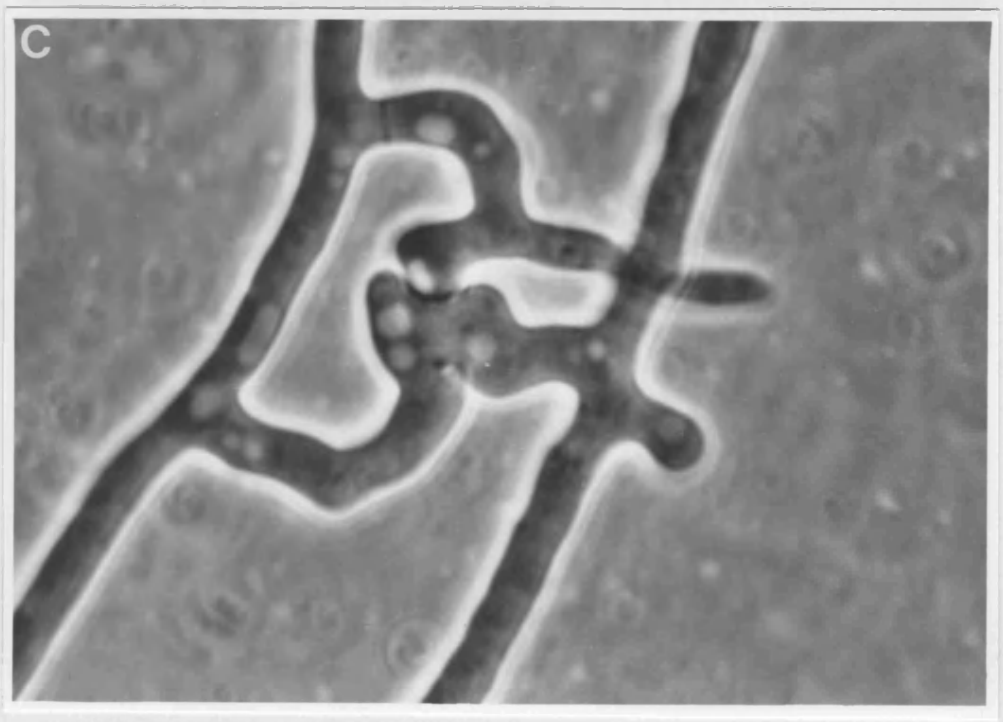

C

Figure 7.3. Sequence of events preceding and during an open pore/cytoplasmic exchange (OPCE) self fusion. (A) Overall layout of hyphae showing position of hyphae **a** and **b** growing towards one another. (B,C,D) Approaching hyphal tips. Note how in (C) and (D) **b** bends towards **a**. (E,F,G) Contact prior to opening of the fusion pore in which hyphal tip **b** swells and flattens expanding the contact interface. (H) A fusion pore forms in the centre of the contact zone. Cytoplasmic granules and vacuoles initially moved from **b** into **a**, but later movement occurred in both directions. (I) 12 h following fusion and the pore is still open (now wider than in (H)). Note how a new septum(s) has formed in **a**, and a branch has grown out from **b**. The hyphae appear to be transparent as visibility has deteriorated due to the length of incubation. Bar marker represents 100 µm (A) and 10 µm (B-I). Time (min) is indicated in the lower left- hand corner of (B) to (I).

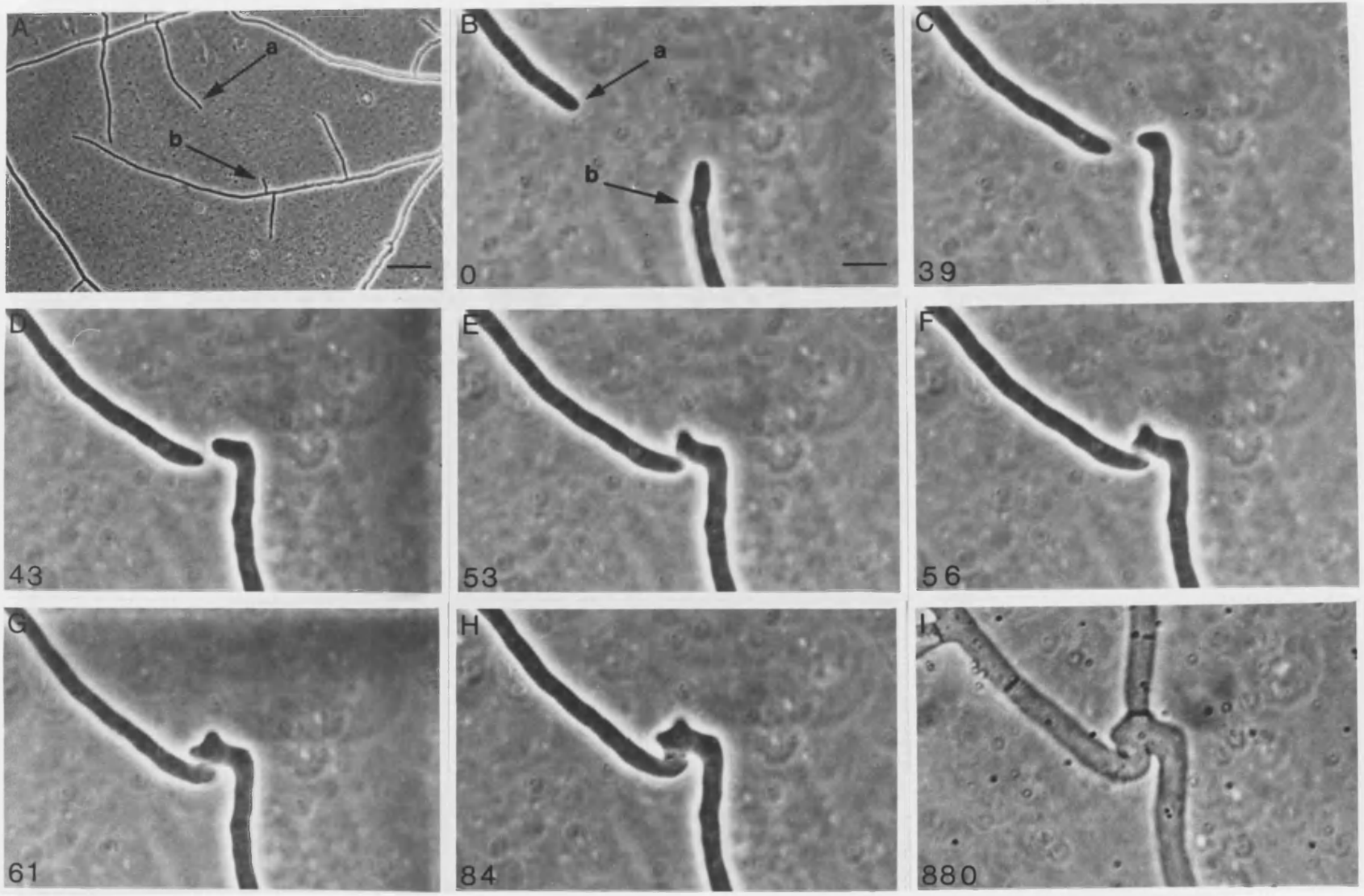
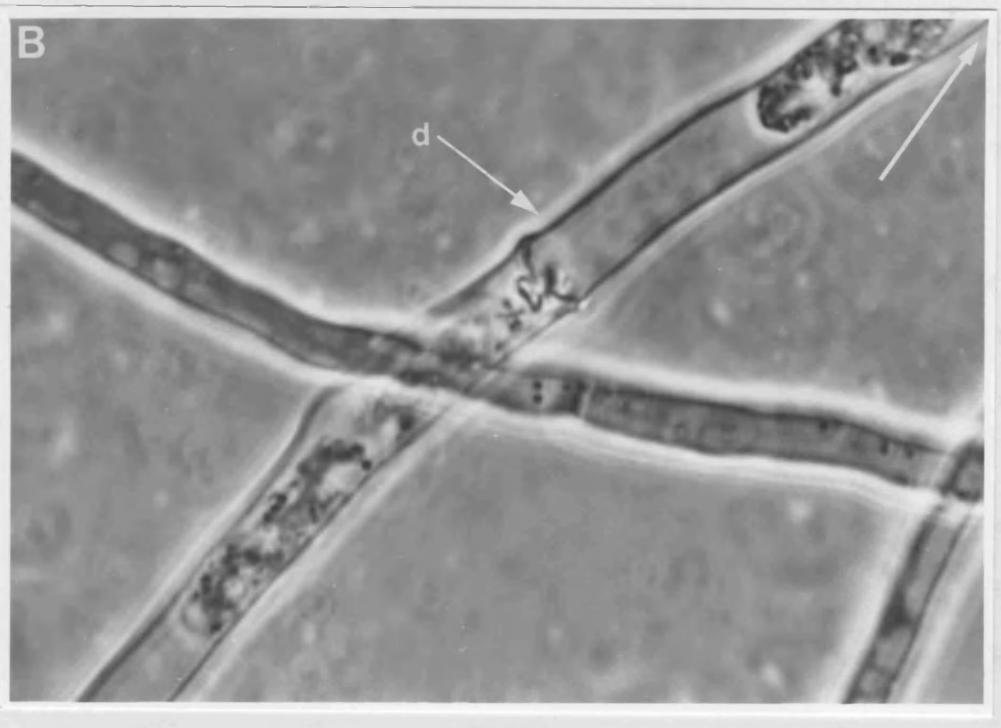

A
a
b
B
a
b
0
C
39
D
43
E
53
F
56
G
61
H
84
I
880

Figure 7.4. Contact/lytic response (CLR) non-self "fusions" between strains **c** and **d** that macroscopically produce a bow-tie reaction when paired in culture on 2% MA. (A) Lateral walls of hyphae of **c** and **d** have been in contact for 6 h. Note how the cytoplasm of one compartment (**1**) of **c** has become very granular and pulled away from the hyphal walls into a "tube". An adjacent compartment (**2**) has become vacuolated and refractile. The lytic response in 2 appears to be at an earlier stage than in **1** (see text). Elsewhere (B) a hypha of **d** has been lysed. The contact zone between **c** and **d** is just out of the field of view (arrowed). Cytoplasm of **d** has become granular, refractile and "rounded off" leaving two compartments mostly empty. This "damage" was evident in three compartments of **d** adjacent to the contact area. Bar marker represents 10 µm.


A
2
c
1
d


B
d

CHAPTER 8

DISCUSSION

8.1 Introduction

The present study has been concerned with gathering information about the mycelial biology of xylariaceous fungi with a view to advancing understanding of their ecology, life cycles, population structure and colonization strategies, about which remarkably little is known. A variety of species have been investigated with respect to the genetically-based variation in their mycelial characteristics, vegetative mycelial transitions between morphologically different forms, their intraspecific and interspecific recognition reactions (and possible occurrence of heterokaryosis) and the distribution of individual genotypes in natural populations. The results of these investigations were discussed in detail in the relevant chapters. However, the diverse themes underlying them are interconnected. The link between these themes can be understood in terms of a framework based on the concept of ecological strategies.

Ecological strategies encompass the behavioural attributes of organisms which enable them to overcome the various constraints that operate to limit their natural distribution (Rayner, Boddy and Dowson, 1987). A spectrum of such behavioural patterns has emerged as a result of different types of selection pressure being exerted throughout the course of evolution. Opposite ends of this spectrum

are marked by two sets of organisms with respect to reproductive commitment and life span. K-selected organisms are characterized by slow or intermittent commitment to reproduction (and the proportion of captured resources they devote at any one time to reproduction is small) and a long individual life span. The converse is true for r-selected organisms (Harper and Ogden, 1970). Analysis of the selection pressures involved has shown that they fall into three fundamentally different forms; competitive stress (C-selection), environmental stress (S-selection) and disturbance (R-selection). According to a scheme favoured by some plant ecologists (Grime, 1977) and fungal ecologists (Pugh, 1980; Cooke and Rayner, 1984), three primary ecological strategies - combative, stress- tolerant and ruderal - result from C-selection, S-selection and R-selection respectively. C- and S-selection can be considered as alternative forms of K-selection, whilst R-selection is equivalent to r-selection. A brief outline of the characteristic features of the three primary strategies and their determinant factors, according to Cooke and Rayner (1984), will follow.

Competition involves the struggle for capture and defence of resources between neighbouring fungi, whilst environmental stress is any form of continuously imposed environmental extreme (e.g. extremes of temperature, aeration, water potential, inhibitory chemicals or lack of available nutrients) which tends to restrict fungal biomass production. Disturbance is the state in which whole or part of the total fungal biomass is destroyed or subjected to new selection pressures by a drastic change in environmental

conditions. Stress factors are a relatively long-lived or permanent feature of some habitats, in contrast to disturbance factors which, while being more severe, are transient.

A combative strategy will maximise occupation and exploitation of resources in relatively non-stressed and undisturbed conditions, whilst a stress-tolerant strategy involves the development of adaptations which allow endurance of conditions of continuous environmental stress. A ruderal strategy, characterized by a short life span coupled with high reproductive potential, determines success in severely disturbed but nutrient-rich conditions. These three primary strategies form a continuum so that where they overlap there are transition zones. Here secondary strategies may emerge combining some of the main characteristics of the adjacent primary strategies. An organism may exhibit combinations of different strategies at any one time or at different times in its life cycle.

Certain characteristic qualities may be expected to be attributed to species that adopt a particular primary strategy. Ruderal species for example depend on early arrival at an unoccupied resource, rapid exploitation of assimilable nutrients and early exit as competitors become established. By contrast stress-tolerant and combative species produce a persistent mycelium. The former however only persist as long as stress conditions are maintained; if stress is alleviated they may be replaced. They do not necessarily have rapid growth, spore

germination or reproduction rates. Combative species may be long-lived and are capable of defending domain gained by primary resource capture or obtaining domain from previous residents by secondary resource capture. They may or may not have rapid growth and spore germination, but generally have slow or intermittent reproduction.

8.2 Colonization strategies

Subordinate to the general concept of ecological strategies, that define the overall behaviour which organisms may exhibit to cope with general problems of survival, are strategies that are concerned with particular problems. Such strategies include those for colonization proposed by Rayner, Boddy and Dowson (1987), by wood-decay fungi that invade the standing tree and fallen timber. Strategies concerned with colonization of the standing tree for example, define the specific tactics that enable fungi to establish themselves by overcoming, circumventing or tolerating the conditions in functional sapwood that are unfavourable to mycelial growth. Five strategies have been postulated in the standing tree: unspecialized opportunism, active pathogenesis, specialized opportunism, heart rot and desiccation tolerance. Like the general ecological strategies, they are part of a spectrum of behaviour, so that an organism may display combinations of different strategies at any one time, or at different times in its life cycle. Indeed colonization and ecological strategies are interrelated. For instance unspecialized opportunism may incorporate ruderal and combative attributes, whilst the remaining four colonization

strategies involve various forms of stress-tolerance. Details of these colonization strategies were outlined in Chapter 1 (Section 1.1, ii, (b)), however they will be repeated here where they apply to particular xylariaceous species.

Several of the xylariaceous species studied during the present investigation appeared to exist as a limited number of genotypes occupying considerable volumes of wood in standing trees. These included Daldinia concentrica, Hypoxylon rubiginosum, "Hypoxylon purpureum" and Hypoxylon nummularium. The large volume of wood occupied by single genotypes seemed to be inconsistent with the mycelial extension rates of these species in culture. Further, close examination of the wood often revealed no obvious colonization courts such as wounds or branch stubs. It was for these reasons that it seems feasible to speculate that these fungi may exhibit, amongst others, stress-tolerant characteristics, establishing themselves via a possible colonization process referred to as latent invasion (Rayner, Watling and Frankland, 1985) - a feature of a specialist opportunist strategy. Although latent invasion has been explained previously (Chapters 1 and 5, Sections 1.1,ii, (b) and 5.4 respectively) it will be outlined again here.

According to the theory of latent invasion the fungus is specialized in its ability to tolerate and initially establish itself in the stressful conditions imposed in functional sapwood, which are considered unfavourable for mycelial growth (Rayner,

1986; Rayner and Boddy, 1986). These conditions include high water content, corresponding restricted aeration and a gaseous phase normally high in carbon dioxide and low in oxygen (Boddy and Rayner, 1983). In response to these conditions, having gained entry to a living tree or branch as a propagule via a minor discontinuity (such as a lenticel, twig or leaf scar), the fungus may develop either as sparse mycelium or as several discrete mycelial units disseminated by the sapstream (such as budding cells or mycelial fragments) (Cooke and Rayner, 1984). The fungus may hence become extensively established in a tree or branch, but not overtly so. Should factors (such as drought or light suppression) subsequently alleviate the stressful microenvironmental conditions imposed in functional sapwood, the fungus may switch to full filamentous mycelial development, causing the decay that is typically associated with loss of sapwood function (Boddy and Rayner, 1983). In this way the fungus capitalizes on events that have occurred independently of its presence, and as such may be regarded as an opportunist.

Other xylariaceous species that colonize standing trees such as Hypoxylon fragiforme and Hypoxylon fuscum usually existed as numerous genotypes each confined to a small domain. The idea of latency may also apply to these species, as it seems to be consistent with the apparently simultaneous appearance of so many genotypes, in the absence of obvious colonization courts. In species such as D. concentrica establishment seems to be effected from within the xylem, presumably a rare event, resulting in a

limited number of extensive genotypes. This may be referred to as endogenous establishment (Rayner and Boddy, in press). However, the existence of several genotypes with relatively small domains in H. fragiforme and H. fuscum, indicates that establishment may have occurred from bases in the bark. Opportunities for establishment in the bark are increased and this is referred to as exogenous etablishment (Rayner and Boddy, in press). It seems reasonable to suggest that numerous genetically different propagules (ascospores) may gain access to the bark of a healthy tree where suitably specialized (stress-tolerant) fungi may be capable of limited saprotrophic survival (Cooke and Rayner, 1984). Once the hostile microenvironmental conditions in the tree (that restrict filamentous development) are relieved, these fungi exploit their position by rapidly colonizing the wood. As this occurs from several individual foci (arising from genetically different propagules) the result is a mosaic of overlapping domains. It should be pointed out that in addition to possible establishment from the bark it seems likely that H. fragiforme can also establish itself from within the xylem (L. Boddy pers. comm.).

A latent invasion colonization process has been suggested previously for certain xylariaceous fungi. Boddy, Gibbon and Grundy (1985), for example, compared colonization patterns of D. concentrica with those of fungal pioneers of attached oak branches and postulated that D. concentrica may invade latently. Following the drought of 1980, the dramatic increase in Hypoxylon atropunctatum canker on oaks (Quercus spp.) in the southern United

States of America was also believed to be attributable to latency (Bassett and Fenn, 1983, 1984).

Other xylariaceous species seem to adopt different colonization strategies. Species such as Hypoxylon mammatum and Rosellinia desmazieresii, that are considered to be pathogenic, presumably colonize their hosts (Populus spp. and Salix repens respectively) when the latter are in a vigorous and healthy condition. Although the distribution of genotypes in wood was not ascertained for either of these species, it is reasonable to suggest that they colonize via active pathogenesis. That is they kill the living tissues of the host, alleviating the hostile conditions of functional sapwood that otherwise restrict their growth (Rayner and Boddy, in press). How these fungi initially gain access to the sapwood is unclear. With regards to H. mammatum several studies have implicated insect wounds (Manion, 1975; Anderson, Ostry and Anderson, 1976, 1979; Anderson and Ostry, 1983) as well as branch stubs and axils (Manion, 1975; Ostry and Anderson, 1979) as mentioned in Chapter 1 (Section 1.1, i, (b)).

A further distinctive colonization strategy may be exhibited by Hypoxylon serpens. Collections of this species were always on decorticated wood found on the woodland floor. This apparent lack of host preference is consistent with previous reports of this fungus (Miller, 1961; Whalley, 1985). Unfortunately, as isolations from wood were difficult to obtain (see Chapter 5, Section 5.3, i, (a)) assessment of the behavioural characteristics of this fungus

(i.e. ecological and colonization strategies) are largely speculative. It seems likely from its lack of host selectivity, occurrence on fallen timber rather than standing trees and the suggestion that individual genotypes may occupy relatively small domains, probably arising from arrival by airborne propagules (Dowson, 1982), that H. serpens exhibits ruderal characteristics. Further, this fungus is considered to be a secondary colonizer (Whalley, 1985). As such it probably displays combative attributes enabling it to gain domain via secondary resource capture; fallen timber rarely arriving on the woodland floor in an uncolonized state (Rayner, Boddy and Dowson, 1987).

Xylaria hypoxylon may exhibit similar ruderal attributes to H. serpens. This species frequently occurs as numerous genetically unique individuals on a wide range of stumps, felled or fallen timber. Usually such individuals each occupy discrete domains clearly demarcated by zone lines (Coates and Rayner, 1985 b, c), although occasionally several genotypes may be present in an apparently uniform decay column (Dowson, 1982). However, the occurrence of X. hypoxylon as a mosaic of individually distinct domains under the bark of a branch, indicates that this fungus may sometimes invade the standing tree exogenously, as described above for H. fragiforme and H. fuscum (A.D.M. Rayner, pers. comm.).

Various other Xylaria species (X. longipes - one sample; X. polymorpha - two samples) were collected in the present study. However, repeated attempts at germinating single ascospores from

these species failed. This also applied to Ustulina deusta (four samples) the cause of butt rot of lime (Tilia vulgaris), elm (Ulmus spp.) and beech (Fagus sylvatica) (Wilkins, 1936, 1939, 1943).

In the above account the emphasis has been on the various ways that the fungi cope with the problems of initially establishing themselves in a resource, be it a standing tree or fallen timber. Establishment is however, only one component of the colonization process. Another important aspect of colonization is that of defence and/or extension of domain, involving the direct interaction between fungi of the same and/or different species. The pincer aerial mycelium reaction (see Chapter 4, Sections 4.3, i, (b) and 4.4) may be significant with respect to intraspecific interactions. If such a reaction occurs in nature it enables individual genotypes to increase their domain at the expense of other genotypes.

With regard to interspecific interactions involving xylariaceous species the present investigation demonstrated that these fungi are relatively combative (see Chapter 6). The usual outcome of direct physiological challenge between different species was an intermycelial reaction resulting in deadlock, in which each participant was mutually excluded from the other's domain, or replacement of one by the other. That these Xylariaceae possess combative ability is perhaps not surprising. As explained previously, many of these fungi may establish themselves in healthy standing trees, possibly exhibiting stress-tolerant characteristics

in order to do so. However, an alleviation of these stressful conditions that select for such fungi, pressures increase for exit, usually via rapid commitment to reproduction (ruderal attributes), or for defence of captured domain (combative attributes). It seems these xylariaceous species fall primarily into the latter category. It should be pointed out that a combative hierarchy was evident so that each species possessed a different combative ability. Daldinia concentrica was the most combative species as it replaced all other species with which it was paired, whilst H. nummularium was the weakest combatant. It may be that weaker combatants may tend to display more ruderal characteristics (such as having the ability to fruit rapidly) than stronger combatants.

8.3 Developmental regulation

It should be clear from the above discussion that the fungal thallus needs to be both versatile and co-ordinated in order to cope with the wide variety of, often conflicting, environmental demands to which it is exposed during the colonization process. These environmental conditions can alter in space and time from the moment of arrival at, establishment in, exploitation and defence of the resource and exit from it. As explained in Chapter 3 (Section 3.1), this versatility of the fungal thallus is thought to arise through the ability of the mycelium to alternate between distinctive morphological and functional modes. Expression of these modes may be regulated genetically by a hierarchical series of switch mechanisms which can be cued by a variety of exogenous and endogenous stimuli (Rayner and Coates, 1987; Rayner, Boddy and

Dowson, 1987). Although developmental versatility of xylariaceous fungi was discussed in Chapter 3, a brief summary of the salient points with regard to colonization will now follow.

The same mycelial genotype of some of the Xylariaceae in the present study demonstrated considerable developmental plasticity, even under the non-selective, homogeneous conditions in artificial culture. For example mycelia of "Hypoxylon purpureum" and Hypoxylon serpens displayed, amongst others, slow-dense/fast-effuse and determinate (unicellular)/indeterminate (filamentous) mode switches. In addition Hypoxylon fragiforme and Hypoxylon multiforme mycelia alternated between juvenile and senescent development.

The ecological significance of a determinate-indeterminate transition lies in the advantage of a unicellular or yeast form in stress-tolerance, and dispersion in mobile media, prior to rapid exploitation by the mycelium, when conditions improve. These are obviously features required for latent invasion. Alterations in internode length and branch angle of hyphae result in either slow-dense (small internodes, wide angles) or fast-effuse (large internodes, smaller angles) modes. These adjustments also have clear implications for the colonization process. The slow-dense mode may aid initial establishment, utilization of nutrients and consolidation of territorial domain, whilst the fast-effuse mode may allow exploration and coverage of domain. The functional role of juvenile (well-developed aerial growth)-senescent (suppressed aerial and/or radial growth) transitions may concern the redir-

ection of growth in preparation for the adoption of a new morphogenetic mode (Rayner and Coates, 1987; Rayner, Boddy and Dowson, 1987).

Abrupt transitions between distinctive developmental states, such as those outlined above, were not evident in culture for all the xylariaceous species studied. For example Daldinia concentrica and Hypoxylon nummularium consistently produced uniform mycelial mats on 2% MA at 20°C in darkness. Nevertheless these fungi probably contain within their developmental programming, certain basic morphogenetic options. It may be that they express these options in a continuum. Alternatively the exogenous and/or endogenous stimuli required to trigger the different mycelial modes, may not have been provided. Nonetheless, mycelia of both these species frequently changed mode along the interaction interface in pairings with mycelia of the same and different species (Chapters 4 and 6 respectively).

In intraspecific pairings non-self rejection seemed to involve activation of senescence pathways (resulting in appressed growth and pigmentation) whilst non-self acceptance seemed to activate juvenile pathways (production of abundant aerial mycelium). Further, rejection in white silky (ws) H. serpens and Hypoxylon mammatum often involved a mode switch between diffuse and compacted morphogenesis, the hyphae becoming aggregated into pseudosclerotial plates along the interaction zone. An alteration such as this, may be significant in relation to defence of territorial domain.

Similarly interspecific interactions resulted in morphogenetic shifts. For example in two pairings with Hymenochaete corrugata, Hypoxylon fuscum hyphae become aggregated together into cord-like structures extending for a short distance into the basidiomycete's domain (Figure 6.3 B). Also D. concentrica often effected replacement of Hypoxylon species by formation of an effuse front of silky-textured white aerial mycelium.

8.4 Population structure and breeding biology

The above discussion of the possible colonization strategies adopted by the various Xylariaceae studied, is based on insights into the spatial and genetic structure of their natural populations. These insights were gained through analysis of the distribution and variation of genotypes (identified on the basis of somatic incompatibility - see Chapter 4) in the field. The structure of a population depends on the pattern of breeding biology adopted. Hence variable populations result from sexual outcrossing, whilst clonal populations composed of genetically identical, or very similar, individuals are the product of sexual non-outcrossing and asexual reproduction. Population structure and breeding biology were the subjects of Chapter 5, however the principal findings reported there will be outlined below.

The species studied fell into two categories with respect to the genetic structure of their populations. The first and largest category, containing eight out of the eleven species examined, had variable populations and appeared to be sexually outcrossing. That

is fertilization in these species invariably seems to occur between genetically different individuals. This was because ascospore progeny from the same perithecium were culturally variable and produced a variety of somatically incompatible reactions when paired together. The second category, containing three species - Hypoxylon multiforme, "Hypoxylon purpureum" and Rosellinia desmazieresii - were composed of a series of somatically incompatible clonal sub-populations and appeared to be predominantly sexually non- outcrossing. Ascospore progeny from the same perithecium in these species were usually culturally similar and somatically compatible.

The spatial structure of populations was analysed by combining evidence from the presence of interactive zone lines in wood and the occurrence of somatic incompatibility between isolates in culture. As this distribution of genotypes depends on whether outcrossing or non-outcrossing predominates, species with these alternate breeding strategies will be considered separately.

In outcrossing species each genotype was restricted to a single resource unit, such as a tree or log, suggesting that ascospores were the agents of infection. It is unlikely that conidia were the infective agents as, if they were, at least a small proportion of trees or logs at the same site would have been expected to be occupied by the same genotype.

As mentioned earlier, in the discussion of colonization strategies, species that attack standing trees either existed predominantly as a limited number of genotypes occupying extensive domains, or as several genotypes occupying relatively small domains. Species in the former group included Daldinia concentrica, Hypoxylon nummularium and Hypoxylon rubiginosum whilst Hypoxylon fragiforme and Hypoxylon fuscum are examples of the latter. Hypoxylon serpens and Xylaria hypoxylon usually occur on detached wood or stumps as numerous individual genotypes each with small domains demarcated by zone lines. However, occasionally X. hypoxylon, for example, may exist in logs as several genotypes in a single uniform decay column (Dowson, 1982).

Of the primarily non-outcrossing species, the fullest investigation was conducted on H. multiforme. This species seems to be divided into numerous somatically incompatible clonal sub-populations. Usually a single genotype occupied a considerable volume of wood in a standing tree. The ascospore progeny from the perithecia this genotype produced were invariably of the same genotype and the product of non-outcrossing. However, at a particular site, different genotypes frequently existed in individual trees, although occasionally the same genotype occurred in more than one tree. For example at Conkwell Wood, Wiltshire, the same genotype was retrieved from two trees 10.67 m apart (Chapter 5, Figure 5.10). Further, although 47 samples from 13 geographical locations were examined, the same genotype was never recovered from more than one site.

This observed population structure in H. multiforme may involve occasional outcrossing. Three perithecia were found to be derived from outcrossing - one from Sweden and two on log AA, from Ashclyst Forest, Devon. It seems likely therefore that in H. multiforme outcrossing will occur if the opportunity arises. Should this opportunity fail to arise, then presumably homomixis (i.e. self fertilization of the haploid homokaryon) or apomixis (i.e. ascospore production without sexual fusion) occurs.

The situation in "H. purpureum" and R. desmazieresii appears to be similar. For example ascospore progeny of "H. purpureum" from the same perithecium were always the product of non-outcrossing. Further, single genotypes occupied large volumes of wood. Where such a genotype corresponded to the position of a particular perithecium, the ascospore progeny from that perithecium were somatically compatible with the genotype from the wood below. However, the same genotype was never recovered from more than one tree or log.

Rosellinia desmazieresii causes death of the dominant shrub-creeping willow (Salix repens) - in the unforested areas of Ainsdale Sand Dunes Nature Reserve, Lancashire; death occurring in rings up to 25 m in diameter (Barrett and Payne, 1982). Ascospore strains derived from perithecia collected from around the circumference of a single ring were found to be of the same genotype and non-outcrossing. However, ascospore strains from different rings were found to be genetically different (at least

with respect to somatic incompatibility loci). It seems reasonable to suggest that a population structure such as this may well arise in a similar manner to that suggested for H. multiforme. That is a sexually non-outcrossing breeding strategy may predominate in the population, but variation may be generated through occasional outcrossing. As the R. desmazieresii strains from each of the rings in the present study were genetically different, it is likely that such rings are primary infection foci. It is possible that an individual genotype may have been recovered from more than one ring had a more extensive study been conducted. Such genetically similar rings would perhaps represent secondary infection foci.

8.5 Stages in the life cycle

With regards to elucidating possible stages in the life cycle of xylariaceous fungi, the present investigation into their mycelial biology has provided evidence that substantiates what was already suspected. It has demonstrated that ascospores are likely to be the prime agents of infection, a view supported by Miller (1961). Indeed Rogers (1967) made the same suggestion following experimental inoculations of Hypoxylon fuscum ascospores in Alnus tenuifolia.

Conidia produced in abundance immediately the ectostroma is exposed (Miller, 1961) or directly on natural substrata, may function as spermatia, as Miller (1961), Rogers and Berbee (1964) and Jong and Rogers (1972) speculated. This view was countered by Cain (1972), who, commenting on evolution among Xylariaceae,

suggested that it was likely that they never possessed spermatia. However, if conidia do not act as spermatia, nor as infective agents, their role remains a mystery. Here they will be regarded as functioning as spermatia, playing a critical role in the life cycle. Spermatia are usually defined as incapable of independent germination. Although conidia, for example of Hypoxylon serpens did germinate in the present investigation, germination was slow (4 d for wf conidia and 7-8 d for gc conidia compared to 1-2 d for ascospore germination - Chapter 3, Section 3.3, ii, (d)). Germination of conidia may also be slow in nature, perhaps supporting their possible role as spermatia.The possible stages of the life cycle will be proposed below combining information obtained from this study, that of Miller's (1961) account of the development of Hypoxylon species in wood and observations by Ingold (1971) on ascospore discharge in Britain. Daldinia concentrica will be used as a model.

Following ascospore germination, D. concentrica may colonize a standing tree via a possible latent invasion strategy (explained above). Through this, a single genotype may come to occupy a considerable domain so that it is able to produce a stroma or stromata. This may occur through adjacent hyphae under and in the bark coalescing together and eventually rupturing the bark. As soon as the ectostroma is exposed, its surface may become covered in conidia and in the periphery of the entostroma below, perithecial initials may form (Miller, 1961).

It was shown that a single genotype in wood produced the sterile tissue of the stroma, and yet the ascospore progeny of such a stroma was outcrossing (Chapter 5, Section 5.3, i, (a)). Hence it seems probable that self-fertilization between conidia (acting as spermatia) and the ascogonia of a particular stroma does not occur. As in certain angiosperms where stamens and carpels may mature at different times, prevention of self-fertilization may be a result of delay in timing between the production of mature conidia and the receptiveness to fertilization of the ascogonium. In species that possess them, trichogynes may be the receptive structure; several authors have reported finding what appear to be trichogynes in some xylariaceous fungi (Rogers, 1979a).

Alternatively, as in self-sterile angiosperms (where a plant's pollen fails to germinate on its own stigmas) it may be that ascogonia are not receptive to spermatia produced on the same stroma. However, fertilization and subsequent ascospore and perithecium development may follow the arrival of wind-borne (or perhaps insect-borne) spermatia derived from a stroma of a different genotype. As most Ascomycotina are dimictic it seems likely that in the Xylariaceae fertilization may occur only between genotypes of complementary mating types. Presumably in primarily non-outcrossing species, such as Hypoxylon multiforme, self-fertilization is not precluded.

Ascospore maturation occurs in May and throughout the summer (autumn for Hypoxylon species - Hodgkiss and Harvey, 1969) they are

discharged nocturnally, normally being shot to a distance of 0.5 - 1.8 cm from the perithecium (Ingold, 1971). These ascospores are probably wind-borne and if by chance a spore gains access to a standing tree the process described above is repeated. It is probable that, because D. concentrica appears to be a relatively persistent species, a single genotype in the wood may produce a fresh crop of stromata each year.

8.6 Selectivity and ubiquity

An intriguing feature of the wood-decaying species of the Xylariaceae studied here is the apparent variation in host-selectivity which they exhibit. This ranges from a very strong association with a single tree species, through to a lack of host preference. For instance Hypoxylon fragiforme and Hypoxylon nummularium display a high degree of selectivity for beech (Fagus sylvatica), in this investigation their perithecial stromata were always associated with this species (Table 2.1). Daldinia concentrica however is not quite so selective. In Britain this fungus has been found to occur on at least sixteen different angiosperm species (Whalley and Watling, 1980). However it exhibits a strong host preference in the south for ash (Fraxinus excelsior) and has even been recorded on ash wood on an abandoned head of a hammer (Whalley and Watling, 1984). Farther north Betula and other hosts are increasingly involved and in Scotland Betula not Fraxinus becomes the preferred host (Whalley and Watling, 1982). By contrast, Hypoxylon serpens does not exhibit host preferences, its

stroma occuring on decorticated wood of a wide range of hardwoods (Miller, 1961; Whalley, 1985).

A similar range of host-preferences is exhibited by other wood-decay fungi. For example strong selectivity is displayed by Inonotus hispidus on ash (Fraxinus excelsior) and Inonotus dryadeus and Stereum gausapatum on oak (Quercus spp.). By contrast non-selectivity is exhibited by fungi such as Chondrostereum purpureum and Stereum sanguinolentum. These colonize a wide range of deciduous and coniferous trees respectively (Rayner and Boddy, 1986).

Two explanations have been proposed by Rayner and Boddy (1986) to account for such variations in host selectivity. One suggested that selectivity may be understood in terms of the interaction between the virulence of the fungus and the resistance of the host tree. Variations in selectivity may represent varying degrees of adaptation towards particular hosts, as for example between fungal plant pathogens and their hosts. Here unspecialized pathogens with wide host ranges invade where active resistance or passive non-susceptibility mechanisms are minimised. By contrast with regards to specialized pathogens, host and pathogen alternately impose strong selection pressures for the respective development of resistance and virulence in each other's populations. This sets up a complex system of co-evolution culminating in highly specific race - variety interactions regulated by recognition or

non-recognition of single genes resulting in susceptibility or resistance.

This explanation however, was rejected by Rayner and Boddy (1986) for two reasons. Firstly many tree decay fungi exhibiting selectivity are saprotrophic and not pathogenic. Secondly there is no definite evidence that trees seal off functional tissues specifically in response to fungal infection, rather than as a general response to damage and aeration (Boddy and Rayner, 1983).

The alternative explanation for the variation in host selectivity between saprotrophic fungi, proposed by Rayner and Boddy (1986), was to understand it in terms of the three primary ecological strategies (ruderal, stress-tolerant and combative) mentioned earlier. According to this, fungi that are apparently non-selective may possess at least some ruderal characteristics, or otherwise be highly combative. With regards to the former, these fungi may colonize habitats that are relatively competitor-free due to recent disturbance. They may then rapidly assimilate readily available nutrients and reproduce before competitors establish themselves. By contrast fungi that exhibit purely combative strategies are characteristic of habitats which are relatively stress and disturbance free. They may be non-host selective as they rely on the possession of highly effective defensive and/or attacking mechanisms to maintain domain and/or replace other fungi.

Host selective fungi may be those that are stress-tolerant.

They may be specifically adapted to tolerate a particular set of microenvironmental conditions, in an undisturbed habitat, that are unfavourable for the production of biomass by the majority of potential colonists. As a result these fungi can establish in a competitor free habitat. Their selectivity may arise as a consequence of individual tree species possessing different types of stressful conditions. Hence, whilst a fungus may be adapted to the conditions that occur in one tree species, they may not be so well suited to cope with conditions of another.

This second explanation for host selectivity was favoured by Rayner and Boddy (1986) and seems to be the most appropriate with respect to those Xylariaceae studied during the present investigation. Hypoxylon serpens seems to fall into the category of a non-selective fungus exhibiting ruderal characteristics (as does Xylaria hypoxylon) whilst the other xylariaceous species that display host selectivity are obvious examples of the stress-tolerant category. As explained above, the initial competitive advantage that these host-selective species may have through their possible latent invasion colonization strategy, may be subsequently sustained by combative mechanisms. These may enable defence, and even extension of already captured domain.

In this study some apparently host-selective xylariaceous species were isolated from non-host trees. For example H. nummularium which is selective for beech (Fagus sylvatica) was isolated from birch (Betula spp.), hazel (Corylus avellana) and ash

(Fraxinus excelsior) (see Chapter 5, Table 5.5). Similarly, "H. purpureum" whose stromata were also associated with beech (F. sylvatica) was once isolated from a small area of birch (Betula pendula) wood (log S - Chapter 5, Figure 5.6 and Table 5.7). These results emphasize the limitations of taking the occurrence of fruit bodies as a direct reflection of mycelial distribution. The apparent selectivity of H. nummularium for beech as a host may therefore indicate that it is only in this tree species that it has the stress-tolerant characteristics enabling it to establish sufficient domain to produce recognizable stromata. This may well apply to other Xylariaceae. Recently, for example, mycelia of Daldinia concentrica and Hypoxylon fuscum were isolated from a sycamore (Acer pseudoplatanus) trunk, despite the absence of their stromata on the surface (A.D.M. Rayner, pers. comm.).

That mycelia of certain apparently host-selective xylariaceous species are more widespread than their fruit bodies suggest, is supported by the well-documented occurrence of these fungi as endophytes (Petrini and Petrini, 1985). For example, anamorphs of Daldinia species and Hypoxylon serpens have been found as endophytes in ericaceous hosts in Britain, including Calluna vulgaris, Vaccinium myrtillus and Erica cinerea (Petrini, 1984). In addition from evergreen shrubs in western Oregon, endophytic isolates included Geniculosporium and Nodulisporium species and some Xylaria anamorphs (Petrini, Stone and Carroll, 1982).

It may be that these endophytic Xylariaceae should not be regarded as belonging to the same populations as the wood-decaying members. They may differ in their breeding biology, as indicated by H. serpens. Two endophytes of this species, H. serpens "Carroll's strain" and H. serpens "Petrini isolate", recovered from Pinus nigra and Mahonia nervosa respectively, as well as H. serpens "Barron's strain" isolated from soil, are reproductively non-outcrossing. In culture these strains produce perithecial stromata yielding homogeneous progeny sets (Jensen, 1981; Petrini and Rogers, 1986; Kenerley and Rogers, 1976). This contrasts with the wood-decaying H. serpens samples collected in the present investigation which are reproductively outcrossing.

8.7 Future research

To conclude this study of the mycelial biology of xylariaceous fungi, the observed developmental plasticity, mycelial interactions and variety of population structures seem to be associated with the various ecological/colonization strategies adopted. Further, the varying degrees of host selectivity may also be related to these strategies. However, many subjects in this investigation have been incompletely studied. Additional research is required to substantiate the findings reported here and to develop new lines of enquiry. Areas worthy of further investigation that relate to the topics already discussed will be outlined below. Other areas have already been mentioned in the relevant chapters and may only be briefly repeated here.

Firstly, the developmental versatility of the mycelium needs to be examined in more detail. Particular attention should be paid to the response of fungal thalli to various moisture and gaseous regimes that mimic the microenvironmental conditions in standing trees and fallen timber. In addition, examination of colony ontogeny in further strains of the species already studied, as well as strains of other species, may increase understanding of the mechanisms and function of developmental plasticity of the mycelium. This in turn may aid interpretation of ecological behaviour.

Secondly, additional studies of mycelial interaction patterns in intraspecific pairings would extend understanding of the basis and genetic regulation of self-non-self recognition. Several aspects of this topic require consideration. For example, to what extent are the interaction types observed here, that are apparently based on the balance between non-self rejection and acceptance, representative of other Xylariaceae and/or Ascomycotina? Further, are these interactions always under polygenic control? Recent work on one xylariaceous fungus - a species of Rhopalostroma - indicates that they are not. Self-non-self recognition in this species seems to be regulated by one gene with a biallelic locus (A.D.M. Rayner, pers. comm.). Additionally, the basis for recognition at the hyphal level needs to be established and the mechanism by which this recognition activates biochemical and developmental pathways that ultimately elicit the interaction response. All these points may also be applied to the recognition phenomena that operate in

interspecific pairings. Also experimental inoculation of standing trees and/or logs with intraspecific and interspecific pair combinations of various strains, may indicate how the reactions recorded here in culture, relate to those that occur in nature.

Thirdly, evidence is required to substantiate the latent invasion colonization process. An experiment involving serial sectioning of healthy beech (Fagus sylvatica) trunks and allowing development of Hypoxylon nummularium was designed to do this. Subsequent testing for somatic incompatibility between genotypes should have revealed if the same genotype occurred in corresponding positions in different sections, and this would have been strong support for latent invasion. Unfortunately one of the replicates of this experiment was vandalized. Hypoxylon fragiforme rather than H. nummularium developed in many of the log sections of the other replicate. As this study has shown, H. fragiforme usually exists as numerous genotypes occupying small domains. Hence it was not surprising that the same genotype was never recovered from more than one section.

Finally, an extension of the survey of population structures to other xylariaceous species would indicate the relative importance of the breeding and colonization strategies observed here. The occasional occurrence of an outcrossing, as well as a non-outcrossing breeding strategy in Hypoxylon multiforme demands detailed examination. Consideration needs to be given to the mechanism or mechanisms that allow the two strategies to be adopted

by adjacent perithecia on the same stroma. This issue focuses attention on the fact that it is not clear where fertilization takes place. Assuming that conidia act as spermatia, are the progeny of each perithecium the product of fertilization by individual conidia (as seems likely from H. multiforme)? Alternatively, or perhaps in addition, is it possible that several perithecia on a particular stroma are fertilized by the same conidium? It may be that a conidium, landing on the surface of a receptive stroma, may germinate and its hyphae ramify over the surface fertilizing several perithecia. Experiments inducing stromatal production on inoculated logs could be designed to test this and would verify or negate the role of conidia in fertilization. Such experiments may also reveal adaptations that prevent self-fertilization and/or promote outcrossing in what appear to be purely outcrossing species, such as Daldinia concentrica.

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APPENDIX I

Site Codes

A1	Ainsdale Sand Dunes Nature Reserve, southwest Lancashire.
A2	Arlington Court, Devon.
A3	Ashclyst Forest, Devon.
B1	Bath University Campus, Avon.
B2	Bathwick Woods, Avon.
B3	Bigwood, Erddig Estate, North Wales.
B4	Britannia Wood, Bangor, Gwynedd.
B5	Browns Folly Nature Reserve, Avon.
C1	Castle Combe, Wiltshire.
C2	Clifford Bridge Woods, Devon.
C3	Clovelly, Devon.
C4	Colerne Woods, Wiltshire.
C5	Conkwell Woods, Wiltshire.
D1	Ddol Uchaf, Raeme, North Wales.
D2	Delamere Forest, Cheshire.
D3	Durham City, Co. Durham.
F	Friary Woods, Avon.
G	Gainford, Co. Durham.
H	Hafod Wood, Erddig Estate, North Wales.
L1	Leigh Woods, Avon.
L2	Long Ashton Park, Avon.
L3	Longleat, Wiltshire.
M	Manor Wood, Bristol, Avon.
N1	Nayland, Suffolk.
N2	North Newbald, Humberside.
O	Onondaga County, United States of America.
S1	Savernake Forest, Wiltshire.
S2	Steps Bridge, Avon.
S3	Sutton Farm, Bow, Devon.
U	Uppsala, Sweden.
V	Venbridge Wood, Devon.
W1	Waterly Bottom, Gloucestershire.
W2	Wetmoor, Chipping Sodbury, Avon.

APPENDIX II
Abbreviations specific to this investigation

1. Strains/isolates

In all cases x is the strain number.

as x	single ascospore derived strains
c x	single conidium isolates
w x	wood derived strains
H x	single ascospore strains of "*Hypoxylon purpureum*" from a sample collected from Hafod Wood, North Wales.
2Y x	single ascospore strains of "*H. purpureum*" from log 2Y from Friary Woods, Avon

2. Mycelial/colonies

am	aerial mycelium	M	mycelial mounds
BC	buff conidial	MBC	mounds of buff conidial mycelium
CZ	concentrically zoned	ML	mycelial mounds with brown liquid droplets
DG	dull green	R	restricted
gc	grey conidial	T	"typical"
HF	honey to isabelline textured mycelium	U	unrestricted
L	buff conidial with brown liquid droplets	WA	white appressed
		WDC	white downy cottony
LICZ	light induced concentrically zoned	wf	white felty
		ws	white silky

3. Light regimes

D	darkness
L	light
L/D	12 h light/12 h dark

4. Fusion types

CLR	contact lytic response
OPCE	open pore/cytoplasmic exchange

APPENDIX III

Radial extension rates of *Daldinia concentrica* and *Hypoxylon nummularium*.

Table A. Radial extension rates (mm d^{-1}) of a sample of single ascospore strains of *Daldinia concentrica* on 2% malt extract agar at 20°C in darkness.

Stroma Cb, perithecium b.

Strain Number		1	2	3	4	5	6	7	8	9	10
Replicate	i	6.37	5.99	6.23	6.64	6.81	6.41	6.29	7.01	6.6	7.28
	ii	6.69	6.23	6.15	7.37	6.73	6.71	6.52	6.46	5.79	7.12
	iii	6.05	-	-	6.35	-	-	-	7.72	5.21	7.11
Mean		6.37	6.11	6.19	6.79	6.77	6.56	6.41	6.74	5.87	7.12
Standard error		0.018	0.011	0.004	0.030	0.028	0.015	0.011	0.027	0.040	0.0005

Stroma Cb, perithecium d.

Strain number		1	2	3	4	5	6	7
Replicate	i	5.66	5.30	7.67	6.39	6.57	6.47	6.31
	ii	6.71	6.64	8.3	6.14	7.2	6.94	6.45
	iii	-	-	-	6.54	-	6.11	6.19
Mean		6.54	5.97	7.99	6.36	6.89	6.51	6.32
Standard error		0.017	0.070	0.031	0.053	0.031	0.039	0.022

Table B. Radial extension rates (mm d^{-1}) of a sample of wood-derived strains of Daldinia concentrica on 2% malt extract agar at 20°C in darkness.

Roman numerals refer to logs collected from Bathwick Woods, Avon (site B2). Subscripts refer to strains isolated from the same log. Arabic numerals refer to logs collected from Friary Woods, Avon (site F).

Strain Code		I	II_1	II_2	III	IV_1	IV_2	IV_3	IV_4	V_1	V_2
	i	7.47	6.21	5.74	5.06	6.94	6.51	5.68	5.95	5.47	5.42
Replicate	ii	6.69	5.95	5.15	6.02	5.87	7.47	5.48	5.99	5.71	6.08
	iii	7.19 6.22	-	5.31	5.9	-	-	-	-	-	-
Mean		6.89	6.08	5.4	5.66	6.41	6.99	5.58	5.97	5.59	5.75
Standard error		0.028	0.013	0.018	0.030	0.054	0.048	0.01	0.002	0.012	0.033
Strain Code		VI	VII_1	VII_2	VII_3	VII_4	VII_5	VII_6	VII_7	VII_8	VII_9
	i	5.53	7.01	7.1	5.92	6.06	6.35	5.99	6.6	6.23	6.81
Replicate	ii	5.83	7.13	7.3	5.68	6.53	5.54	5.9	6.54	6.24	6.6
	iii	5.27	-	5.83	-	-	-	-	-	-	-
Mean		5.54	7.07	6.74	5.8	6.3	5.95	5.95	6.57	6.24	6.71
Standard error		0.016	0.00003	0.002	0.012	0.024	0.041	0.005	0.003	0.0005	0.011

Table B. (continued).

Strain Code		VII_{10}	VII_{11}	VII_{12}	VII_{13}	VII_{14}	VII_{15}	VII_{16}	VII_{17}	VII_{18}	VII_{19}
	i	5.7	6.57	6.85	6.33	5.95	5.7	5.77	6.9	6.38	5.92
Replicate	ii	5.72	5.76	7.53	6.3	6.17	7.18	4.64	6.09	6.78	5.05
	iii	-	-	-	-	-	-	-	-	-	5.71
Mean		5.71	6.17	7.19	6.32	6.06	6.44	5.21	6.5	6.58	5.56
Standard error		0.001	0.041	0.034	0.002	0.011	0.074	0.057	0.041	0.02	0.026
Strain Code		VII_{21}	VII_{23}	VII_{24}	VII_{25}	VII_{26}	VII_{27}	VII_{28}	VII_{29}	VII_{30}	$VIII_{1}$
	i	5.3	6.12	6.42	5.83	7.45	5.67	6.03	5.42	7.4	5.89
Replicate	ii	5.6	5.25	6.32	5.97	6.15	5.93	6.17	6.25	6.5	6.33
	iii	5.52	-	-	6.39	-	-	-	-	5.8	6.28
Mean		5.47	5.69	6.37	6.06	6.8	5.8	6.1	5.84	6.6	6.17
Standard error		0.009	0.044	0.005	0.017	0.065	0.013	0.007	0.042	0.046	0.014

Table B. (continued).

Strain Code		$VIII_2$	$VIII_3$	1	2	3	4
	i	7.11	6.2	6.5	5.67	4.94	5.94
Replicate	ii	7.0	6.65	6.59	5.08	5.42	5.75
	iii	7.31	-	-	-	-	-
Mean		7.14	6.43	6.55	5.38	5.18	5.85
Standard error		0.009	0.023	0.005	0.03	0.024	0.010

Table C. Radial extension rates (mm d^{-1}) of a sample of single ascospore strains of Hypoxylon nummularium on 2% malt extract agar at 20°C in darkness.

Strain Number		1	2	3	4	5	6	7	8	9	10
	i	6.9	6.8	7.8	6.8	6.7	7.5	6.6	7.5	7.9	8.4
Replicate	ii	7.3	7.0	7.3	-	6.4	8.1	6.7	7.3	7.9	8.5
	iii	7.0	7.2	7.6	6.9	6.6	7.6	6.8	8.1	7.0	8.0
Mean		7.1	7.0	7.6	6.8	6.6	7.8	6.7	7.7	7.6	8.3
Standard error		0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.03	0.01
Strain number		11	12	13	14	15	16	17	18	19	20
	i	8.8	8.1	7.7	7.1	7.8	7.4	7.2	7.5	6.2	7.2
Replicate	ii	7.5	8.4	9.1	7.1	7.6	6.9	6.9	7.4	6.5	7.4
	iii	7.8	7.2	8.6	7.3	7.3	6.9	7.0	7.4	6.6	7.2
Mean		8.0	7.9	8.5	7.2	7.6	7.1	7.1	7.4	6.4	7.3
Standard error		0.04	0.03	0.04	0.01	0.01	0.02	0.01	0.002	0.01	0.01

Trans. Br. mycol. Soc. **86** (4), 643–649 (1986) *Printed in Great Britain*

MYCELIAL INTERACTIONS IN *DALDINIA CONCENTRICA*

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Genetically different isolates of *Daldinia concentrica* always produced demarcation zones when paired against one another. The morphology of these zones varied with respect to the width and shape of regions of white aerial mycelium (*wam*).

In pairings between single ascospore isolates from the same perithecium, evidence was obtained for a multigenic recognition system. The *wam* usually widened, either symmetrically or asymmetrically, resulting in 'bow-tie' and 'hour-glass' shaped interaction zones. Bow-tie shaped zones were associated with inhibition of the parent colony margins and emergence of a fan-shaped *wam*.

Pairings between isolates from different stromata and from different wood samples generally resulted in a narrower *wam* of more constant width at the interaction interface. However, a large number of unilateral 'pincer-like' interactions occurred in which one isolate gained territorial dominance over the other. These interactions followed outgrowth of a *wam* from the interaction interface associated with inhibition of extension of the suppressed isolate.

Evidence that the *wam* contained temporarily heterokaryotic mycelium was obtained by subculturing single hyphal tips.

These observations imply the existence of mycelial recognition systems which are fundamentally similar to those governing mating in heterothallic Basidiomycotina, but which do not enable production of a stable secondary mycelium.

Xylariaceous Ascomycotina are a world-wide group of fungi commonly associated with the decay of woody substrata in a varied range of ecosystems. It is therefore remarkable that many aspects of their ecology, life cycles and population structure remain obscure. Work with wood-decaying Basidiomycotina has shown that analysis of interaction patterns between different genotypes can yield useful information about their population biology (Rayner *et al.*, 1984). Recent studies of Ascomycotina, such as those by Anagnostakis (1984) and Brasier (1984) on *Endothia parasitica* (Murr.) And. and *Ceratocystis ulmi* (Buism.) C. Moreau indicate that the same principle applies to these organisms. We describe a study of mycelial interactions in the familiar xylariaceous species, *Daldinia concentrica* Ces. & de Not.

MATERIALS AND METHODS

General isolation and cultural procedures

Unless otherwise specified below, cultures were grown on 20 ml 2% (w/v) malt extract agar (MA) in 9 cm plastic Petri dishes incubated at 20 °C in darkness.

Samples of decaying wood and perithecial stromata of *D. concentrica* were collected from the following eight locations (National Grid reference, type of wood and number of logs sampled respectively are given in parentheses for each site): Bath University Campus, Avon (ST 7764; *Fraxinus excelsior* L., ash; 2); Bathwick Woods, Avon (ST 7764; ash; 8); Clovelly, Devon (SS 3225; ash; 1); Durham City. Co. Durham (NZ 284423; ash; 1), Friary Woods, Avon (ST 7858; ash; 4) Friary Woods, Avon (ST 7858; *Fagus sylvatica* L., beech; 1); Gainford, Co. Durham (NZ 185166; ash; 1); Long Ashton Park, Bristol, Avon (ST 5572; beech; 1); Nayland, Essex (TL 3497; ash; 1); North Newbald, Humberside (SE 9137; ash; 1). Isolates of *D. concentrica* were then obtained as follows.

Fragments, approx. 10 mm^3, cut from wood which had been surface-sterilized in 5% domestic bleach (Parazone) for 5 min were transferred to MA plus 0·01% (w/v) novobiocin plates and incubated until sufficient mycelium had grown out to enable subcultures to be made.

Stromata of *D. concentrica* were wiped with cotton wool soaked in 70% ethanol, broken open to expose fresh tissue, small pieces of which approx. 10 mm^3, were excised and placed on MA plus 0·01%, novobiocin plates and incubated as above.

Single ascospore cultures were obtained from perithecia by using the spore-tendrils or cirrhi which occur when the normal ascus discharge fails. A single cirrhus was picked off, using a sterile needle and transferred to a drop of a filter sterilized 0·01% (w/v) aqueous solution of ampicillin. After mixing, the drop was spread across a series of MA

plus 0·01 % novobiocin plates which were then incubated at 15° in darkness. Single germlings were then transferred to fresh plates using a fine tungsten needle.

Cultural characteristics and experimental pairings

The cultural properties of each isolate were recorded using the descriptive terminologies of Rayner (1970) and Stalpers (1978). Radial extension rates of some isolates were measured along two diameters drawn at right angles on three replicate 14 cm plates containing 75 ml 2 % MA incubated in darkness at 20°.

Experimental pairings were made by placing 6 mm diam disks of inoculum, cut from the margin of actively growing colonies, up to 1 cm apart near the centre of the plates. Cultures were incubated for up to 35 d and examined at regular intervals.

Testing for heterokaryosis in interaction zones

A characteristic feature between interacting isolates was the formation of regions of white aerial mycelium (*wam*). To test for heterokaryosis in this region between paired single ascospore isolates from the same perithecium, the method for hyphal tip isolation (Butler, 1984) was followed. A small piece of mycelium was removed and transferred to a sterile Cellophane disk overlying Czapek-Dox agar containing 2 % starch, and incubated for 2–3 d at 20° in darkness. On this substratum the margin of the resulting colony was diffuse enough to allow excision of a square of Cellophane carrying a single hyphal tip, and for this to be transferred to the centre of a fresh MA plate. When the colony was well established, inocula of the two original strains were placed on either side of it, on the same diameter at the edge of the plate and the resulting interactions between the three colonies recorded.

RESULTS

Cultural characters

All isolates were initially white and cottony to cotton-woolly and had an appressed, even margin. In all single ascospore cultures, and a proportion of

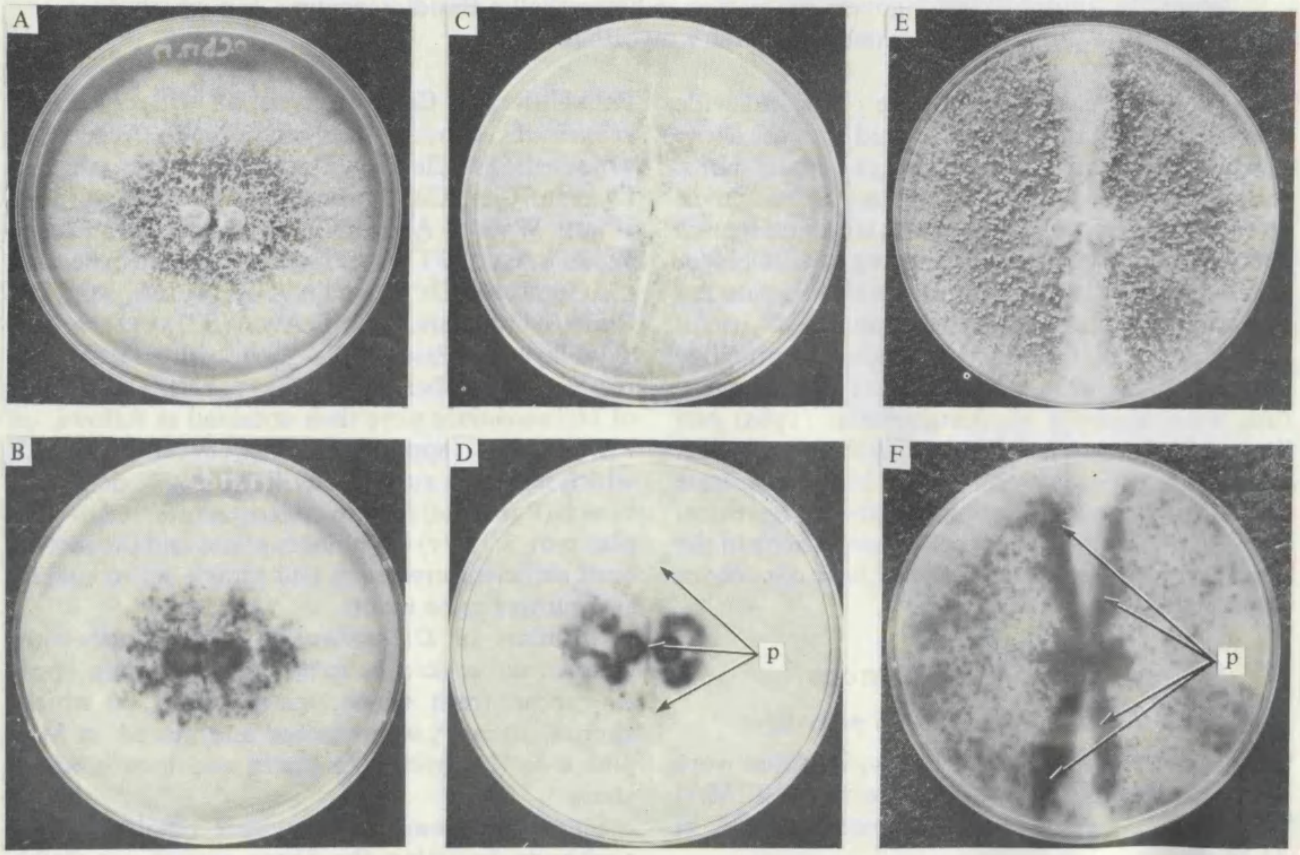

Fig. 1. Interactions between isolates of *Daldinia concentrica*. (A, B) Intermingling (A viewed from above, B viewed from below). The original zone of contact between the two colonies is indiscernible. Note the woolly, granular texture and flecked appearance of the mycelium. (C, D) Narrow line (C from above, D from below). A narrow ($\leqslant 3$ mm) line of woolly-white aerial mycelium (*wam*) is discernible between the colonies, underlain by an amber pigmented zone (p). (E, F) Wide band (E from above, F from below). A wide (>3 mm) zone of more or less constant width containing a *wam* is bounded by fuscous black pigmented regions (p).

wood and stromatal tissue isolates, colonies then darkened to pale olivaceous grey or olivaceous grey and developed a woolly, granular texture with numerous minute olivaceous grey to iron-grey coloured flecks (Fig. 1A, B). Some cultures developed a floccose texture due to development of white mycelial tufts. The remainder of the wood and stromatal isolates became zonate and developed a felty vinaceous buff to hazel-coloured conidial mat. Viewed from below, as the woolly-granular colonies aged, a honey to isabelline to dark mouse grey/fuscous black colouration spread outwards from the inoculum. However, less darkening was observed below felty colonies. Radial extension rates varied from 5·9 to 8·0 mm d^{-1} in single ascospore isolates and from 5·2 to 7·2 mm d^{-1} for wood isolates.

Interaction types

Interactions between isolates of *D. concentrica* were classified into distinctive types as indicated below. A prominent feature in most types was the development of regions of woolly white aerial mycelium (*wam*) in the interaction zone. These regions contained hyphae which were straighter and wider than normal with acute-angled branches.

Intermingling (Fig. 1A, B). The original zone of contact between the colonies disappeared as the morphology of the mycelial mats became uniform, corresponding with the morphology of unpaired isolates.

Narrow Line (Fig. 1C, D). A narrow ($\leqslant 3$ mm) line of demarcation was discernible between the colonies. In 'strong' interactions this contained a *wam* and was amber underneath. In 'weak' interactions neither *wam* nor pigment were present.

Wide Band (Fig. 1E, F). A wide (>3 mm) zone of more or less constant width developed between the colonies. The zone contained a *wam* and was bounded underneath by fuscous black to dark mouse grey pigmented regions. Towards the centre of the demarcation zones amber pigment was often present, and this pigment was also characteristic at early stages of development of the interactions.

Bow-tie (Fig. 2A–C). Bow-tie shaped demarcation zones containing *wam* widening outwards from the centre developed between the colonies. At early stages of development when growth covered approximately half the diameter of the plate, a narrow zone of appressed sparse mycelium separated the colonies. Subsequently a fan-shaped *wam* grew out from the ends of this narrow zone, associated with restriction of extension of the parent colony margins, which contained narrow, densely and obtusely branched hyphae with short compartments as well as numerous hyphal knots and coils. As a result, the *wam* widened either symmetrically or asymmetrically as time passed and they became delimited at their junction with the parent colonies by formation of zones containing amber or luteous pigment, darkening to dark mouse grey or fuscous black.

'Pincer' (Fig. 2D–F). These were an apparent variant of bow-tie interactions in which only one of the parent colony margins was suppressed, resulting in the unilateral outgrowth of *wam*. With time the *wam* tended to develop a morphology similar to that of the non-suppressed colony, which consequently encircled the suppressed colony in a pincer-like movement. Unlike symmetric or asymmetric bow-ties, pincer reactions were fully delimited by formation of pigmented zones only at one side of the pairing, next to the suppressed colony. In pincer interactions involving a 'felty' and a woolly colony type (see above), the woolly type was normally dominant.

Hour-glass (Fig. 2G–I). Here symmetric or asymmetric *wam* delimited by pigmented zones were produced which looked like bow-ties except that they were constricted both at their centre and at their periphery. The reason for the difference in shape from bow-ties appeared to be that the parent colony margins were not, or were only temporarily suppressed.

Distribution of interaction types in experimental pairings

The pattern of occurrence of different interaction types in experimental pairings is shown in Table 1.

Intermingling was only observed in self-pairings and exceptionally between ascospore isolates from the same perithecium. Hour-glass interactions predominated between ascospore isolates derived from the same perithecium or perithecial stroma and were rare (and then only narrow) or absent between isolates from different sources. Conversely, narrow-line and wide-band reactions were frequently recorded in pairings between isolates from different sources, with narrow-line reactions being more frequent between isolates from different geographic locations. The number of bow-tie and pincer reactions did not appear to differ appreciably between pairing types. It should be pointed out that different interactions were sometimes seen when experiments were repeated, perhaps because of changes in colony development patterns during laboratory culture. It may be best, therefore, not to regard differences between interaction types as absolute distinctions.

Genetic designation of hyphal tips from interaction zones

Colonies arising from hyphal tips obtained from the interaction zone between paired isolates normally

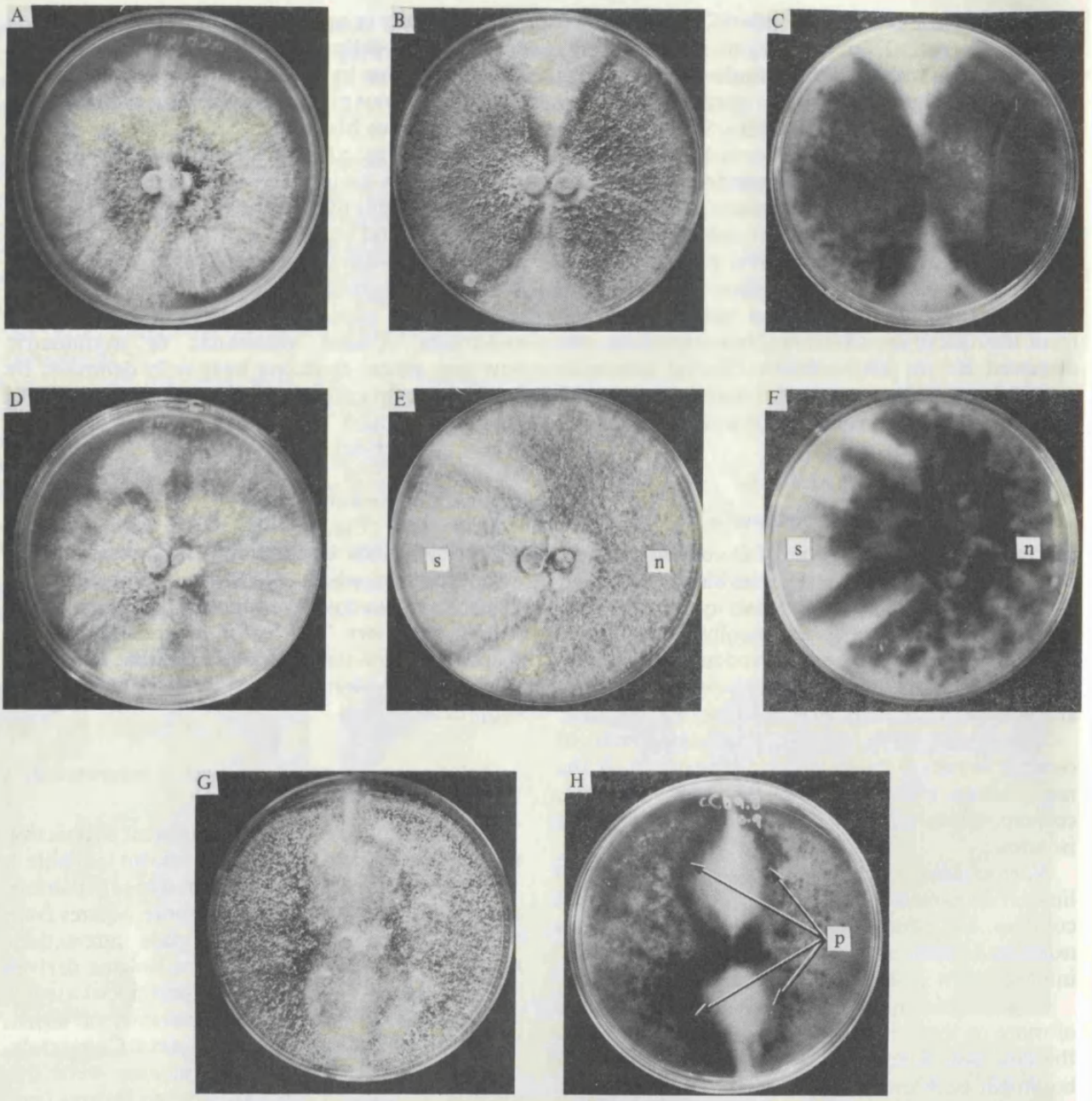

Fig. 2. Interactions between isolates of *Daldinia concentrica*. (A–C) 'Bow-tie'. Early in development (A) the parent colony margins are inhibited allowing emergence of a fan-shaped *wam*. In (B) and (C) (viewed from above and below respectively) a symmetric bow-tie has been produced as a result of restriction of the parent colony margins to an equal extent. (D–F) 'Pincer'. Early in development (D) extension of only one of the parent colony margins is suppressed, resulting in unilateral outgrowth of the *wam*. In (E) and (F) (viewed from above and below respectively) the fully developed pincer shows the non-suppressed isolate (n) has gained territorial dominance, partially encircling the suppressed colony (s). (G, H) Hour-glass (G from above, H from below). A symmetric *wam*, constricted at both its centre and periphery and delimited by fuscous black pigmented regions (p) is visible between the colonies. The isolates paired in (A) and in (D) are different from these paired in (B) and (C), and (E) and (F) respectively.

grew out uniformly and intermingled with one of the two types originally paired, whilst producing a reaction zone against the other. Hence they could be assigned genotypically to one or other of the originals. Sometimes, however, the colonies either grew out uniformly but interacted against both originals, or they produced a *wam* type mycelium which sectored into the two original isolates. Genotype assignment, based on these reactions, of tip cultures obtained from a representative set of pairings between ascospore isolates from the same perithecium is shown in Table 2. The genotype

Table 1. *Occurrence of different interaction types in experimental pairings*

Pairing type	Number of interactions tested	Number of interactions showing					
		Intermingling	Narrow line	Wide band	Bow-tie	Pincer	Hour-glass
Self	176	176	0	0	0	0	0
Intraperithecial	976	8	43	15	125	255	530
Interperithecial							
Same stroma	200	0	1	0	30	39	130
Different stromata							
same site	100	0	9	4	59	26	2
different sites	100	0	39	9	28	24	0
Wood/stromatal isolates							
Same site	83	0	24	30	5	24	0
Different sites	88	0	33	10	12	33	0

Table 2. *Genetic designation of hyphal tips from interaction zones*

Interaction type	Genotypes paired*	Source of hyphal tip†	Genotypes recovered after (d)*				
			7	14	21	28	35
Hour-glass	3, 1	I	1	3	1	3	1
		O	3	3	3	3	3
	3, 2	I	3	2	3	3	2
		O	3	B	3	3	3
	4, 2	I	4	B	2	2	2
		O	B	2	4	B	B
	5, 1	I	1	5	B	5	B
		O	B	5	B	1	1
Bow-tie	2, 1	I	1	2	1	2	B
		O	2	1	2	1	2
	4, 1	I	1	1	1	1	1
		O	4	1	4	1	1
	5, 2	I	5	5	5	5	2
		O	2	2	5	5	2
Pincer	4, 3	I	3	4	4	3	B
		O	3	3	3	3	3
	5, 3	I	3	3	5	3	3
		O	3	5	5	3	3
	5, 4	I	4	B	4	5	5
		O	4	4	B	4	4

* Genotypes are assigned by isolate number. B indicates that both were recovered.
† Hyphal tips were obtained either from I (between inoculum plugs) or O (2 cm away).

recovered varied with time and position of isolation, except in pincer reactions where the dominant genotype was the one recovered after prolonged incubation from the outer parts of interaction zones.

DISCUSSION

It has been suggested that the genetic mechanisms regulating mating in Ascomycotina and Basidiomycotina are so fundamentally different that no parallels can be expected between them (Ullrich, Novotny & Specht, 1985). However, this view may partly reflect the fact that understanding of the relationship between the recognition systems regulating mycelial interactions and mating in these fungi has been obscured by the different criteria chosen for scoring these features.

Thus, mating in heterothallic Basidiomycotina (except Hemibasidiomycetes) is most often identified by the development of a distinctive mating-type heterokaryon or secondary mycelial phase (typically a dikaryon bearing clamp connexions) between paired, homokaryotic isolates derived from single

basidiospores. These mating systems have been found to depend on one, two or exceptionally three multiallelic mating factors, and in some species, in which homokaryotic fruiting occurs, to operate independently of basidium formation.

By contrast, in heterothallic Ascomycotina the formation of independent mating-type heterokaryons does not normally occur, except in secondary homothallic forms, and mating is usually scored on the basis of formation of asci containing viable ascospores. These mating systems have invariably been found to be based on a biallelic incompatibility locus. Heterokaryosis in these fungi is usually detected experimentally by combining strains carrying different nuclear marker genes and scoring for whether or not these markers become associated. Most recent evidence has suggested that heterokaryosis in these fungi is restricted by somatic (=vegetative) incompatibility systems causing rejection of non-self before or soon after hyphal fusion (Anagnostakis, 1984; Brasier, 1984).

In Basidiomycotina any such non-self rejection between different homokaryons must be prevented or overridden if the formation of a heterokaryotic mycelium, which in these somatogamous organisms is imperative for outcrossing, is to be achieved. It has therefore been suggested that the multiallelic mating systems of Basidiomycotina achieve this by a mechanism which allows formation of a stable heterokaryon following a genetically regulated process of access of non-self nuclei into an acceptor mycelium (Rayner *et al.*, 1984).

In the present work, the variability and interactions which occurred between ascospore progeny from the same perithecium suggests that *D. concentrica* is heterothallic, but because perithecia were not formed in culture it was not possible to determine the mating system. The scarcity of intermingling interactions between the isolates, which were probably due to obtaining sister spores from the same ascus, provided evidence that the strong somatic reactions occurring in the remaining combinations were based on a multigenic mechanism.

Interactions between isolates from the same perithecium were predominantly of the bow-tie, hour-glass or pincer type, and hyphal-tip subculturing showed that the interaction zones contained a temporary heterokaryon resulting from donor nuclei being allowed access into an acceptor mycelium and consequently an eventual territorial gain. This is interesting because the interactions were strongly reminiscent of reactions associated with access of non-self nuclei during mating in Basidiomycotina (Coates & Rayner, 1985*a*) in their shape, the inhibition of marginal extension, and in the production of a non-sporulating woolly mycelial phase with straight, wide hyphae and narrow-angled branching patterns. Indeed, the only real differences from Basidiomycotina were the lack of formation of a stable heterokaryon and occurrence of the reactions between all combinations, indicating a multigenic system, rather than between distinctive sets of isolates as occurs in Basidiomycotina with uni- or bifactorial mating systems. Since bow-tie, hour-glass and pincer reactions appeared to be slightly different expressions of basically the same interaction phenotype (partly because, as has been found in Basidiomycotina, developmental status affected outcome) this suggests a version of multiallelism in which variants of a recognition factor occur at several or numerous loci, rather than at the one or two loci found in the multiallelic uni- or bifactorial systems typical of the Basidiomycotina.

Besides the similarity between *D. concentrica* mycelial interactions and certain basidiomycete mating reactions, another possible common feature was the apparent reduction of access, manifested by an increased proportion of wide-band and strong narrow-line reactions, between isolates from different locations. This suggests an inverse relationship between the rate of expression of access and of somatic rejection in a way which has been clearly demonstrated in the basidiomycete *Thanatephorus cucumeris* (Frank) Donk (*Rhizoctonia solani* Kuhn) (Anderson, 1984), a fungus whose production of heterokaryotic tufts of mycelia between mating-compatible homokaryons is also very reminiscent of *wam* production in *D. concentrica*.

Apart from the occurrence of biallelic mating systems in Ascomycotina as opposed to multiallelic mating systems in Basidiomycotina, another reason for doubt about the equivalence of mating systems in these two major groups of fungi has been the demonstration of a 'cassette' mechanism allowing switching of mating types in 'homothallic' strains of the yeast, *Saccharomyces cerevisiae* (Herskowitz & Oshima, 1981; Ullrich *et al.*, 1985). However evidence of such a system has now been found in the Homobasidiomycete, *Stereum hirsutum* (Willd.: Fr.) S. F. Gray (Coates & Rayner, 1985*b*). If the biallelic mating factors and multiallelic mycelial recognition factors of Ascomycotina became physically or functionally integrated, this might provide a basis for basidiomycete mating behaviour.

Finally, it is important to ascertain whether the interactions reported here for *D. concentrica* apply also to other Ascomycotina. There is some evidence that this may be so. For example, 'tuft' mycelia occur between interacting strains of *Ceratocystis ulmi* (Brasier, 1984) and of *Pyricularia oryzae* in which evidence of heterokaryosis in these regions has been provided (Fatemi & Nelson, 1978). In our

own work we have observed mycelial interactions paralleling those of *D. concentrica* in *Hypoxylon nummularium*, *H. fragiforme*, *H. fuscum* and a *Diatrype* sp. (Sharland & Rayner, unpubl.).

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(*Received for publication 7 October 1985*)

Fr., *Panus operculatus* Berk. & Curt., *Pleurotus ostreatus* Fr., and *Schizophyllum commune* Fr. The culture of *Cyathus* was provided by R. J. Bandoni (Univ. of British Columbia), and the others were isolated from spores.

Growth studies were performed on a medium (MYP) consisting of 7 g malt extract, 1 g peptone, 0·5 g yeast extract, and 1 l water. For semi-solid media 15 g agar l^{-1} was added. The water potential of the medium was controlled with KCl (agar media) or polyethylene glycol 4000 (PEG) (liquid media). The amount of KCl required to achieve a particular water potential (Ψ) was calculated from the equation:

$$\text{g KCl } l^{-1} \text{ of MYP} = (\Psi - 0{\cdot}14)\ 1{\cdot}595,$$

where Ψ = the absolute value of the desired water potential in MPa. This equation was generated from a standard curve prepared using a Wescor HR-33T dewpoint microvoltmeter. MYP lacking KCl has a Ψ of $-0{\cdot}14$ MPa. The amount of PEG added for different water potentials was calculated from the formula of Kidd, Reid & Davidson (1972) and checked with the dewpoint microvoltmeter. Agar cultures at different water potentials were started by centrally inoculating 9 cm Petri dishes with agar plugs cut from the margin of actively growing colonies on MYP. Growth was determined by measuring the rate of hyphal extension during the linear phase of growth (days 2–12). Treatment plates were run in triplicate, and experiments were repeated at least once. Plates were incubated at 27 or 20 °C, the latter for *Calocera* and *Dacrymyces* sp. which grew poorly at the higher temperature. For dry weight studies, 125 ml flasks containing 25 ml of MYP (including PEG if added) were inoculated with a blended hyphal suspension and incubated at 27° for 8 days. Each treatment was replicated five times, and the experiment repeated once.

Only *S. commune* made measurable growth at water potentials below -4 MPa (Figs 1, 2), with maximum growth on KCl amended agar occurring at $-3{\cdot}5$ MPa, and 34% of maximum at -6 MPa, the lowest Ψ tested using KCl as the osmoticant. In liquid culture with PEG, this species displayed maximum growth at -1 MPa, 27% of maximum at -6 MPa, and 14% of maximum at -7 MPa (Fig. 2). Seven of the 9 species made maximum growth at water potentials of $-0{\cdot}5$ MPa or higher. *Clitocybe aurantiaca* and *Dacrymyces* sp. were especially sensitive to water stress, with growth optima of $-0{\cdot}14$ MPa and no growth at $-1{\cdot}5$ MPa or lower. After *S. commune*, *M. scorodonius* was most tolerant of water stress, making maximal growth at $-1{\cdot}0$ MPa and measurable growth as low as $-3{\cdot}5$ MPa.

Although KCl was the only osmoticant tested with 8 of the 9 species examined in the present study, previous studies (Boddy, 1983; Wilson & Griffin, 1979) indicate that the general results obtained with this solute, including the slope of the declining growth portion of the curve, are representative of those obtained with other osmoticants.

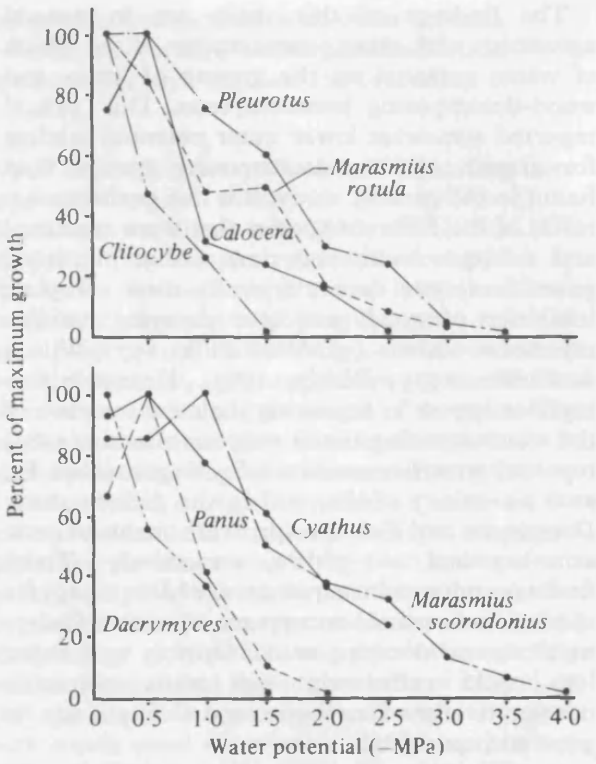

Fig. 1. Effect of water potential on hyphal extension growth of wood- and litter-decay basidiomycetes.

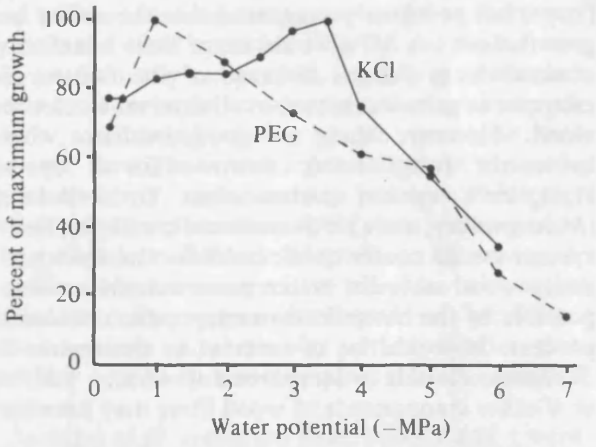

Fig. 2. Effect of water potential on growth of *Schizophyllum commune*. Dry weights of 8-day-old colonies were used to plot the response to water potential controlled by PEG, and linear extension rates on agar were used to plot the response to water potentials controlled by KCl.

The findings of this study are in general agreement with other recent studies of the effects of water potential on the growth of litter- and wood-decomposing basidiomycetes. Dix (1984*b*) reported somewhat lower water potential minima for growth of litter-decomposing species than found in the present study, but this probably is a result of the different species that were examined and a longer incubation time. Other published growth-response curves typically show complete inhibition of wood- and litter-decaying basidiomycetes at −2 to −4·5 MPa (Griffin, 1977; Wilson & Griffin, 1979; Boddy, 1983). Heterobasidiomycetes appear to be among the most sensitive of the wood-decaying basidiomycetes. Boddy (1983) reported growth cessation of *Exidia glandulosa* Fr. at −2·2 to −3·1 MPa, and in the present study *Dacrymyces* and *Calocera* spp. were unable to grow at −1·5 and −2·5 MPa, respectively. These findings support the explanation of Dix (1985) for the late successional occurrence of certain Dacrymycetales on decaying wood. Only in well-rotted logs could sufficiently high water potentials develop to allow *Dacrymyces* and *Calocera* spp. to grow and sporulate.

S. commune, a species characterized by the xerophytic adaptations of its sporophores, is unusual as a wood decay organism in its ability to grow at osmotic potentials below −7 MPa. Griffin (1977) has previously suggested that the ability to grow below −4 MPa would be of little benefit to a wood-decay fungus because of the failure of enzymes to gain access to the cellulose molecules in wood. However there is good evidence that brown-rot fungi break down cellulose by a H_2O_2/Fe^{2+} system rather than by cellulase (Montgomery, 1982). Possession of the H_2O_2/Fe^{2+} system would confer on *S. commune* the ability to decay wood at lower water potentials than those possible by the better-known enzymatic (cellulase) process. It would be of interest to determine if *S. commune* is able to decay wood at −6 to −7 MPa or if other components of wood litter may provide the energy source at water potentials below −4 MPa. Although *S. commune* presently appears to be the wood-decaying basidiomycete most tolerant of water stress, several soil-inhabiting basidiomycetes are able to grow at water potentials below −10 MPa (Wilson & Griffin, 1979).

We thank N. J. Dix for encouragement in this study and for his helpful comments and Chris Nerone for preparing the graphics.

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MYCELIAL DIMORPHISM, INTERACTIONS AND PSEUDOSCLEROTIAL PLATE FORMATION IN *HYMENOCHAETE CORRUGATA*

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Mycelia of *Hymenochaete corrugata* exhibited two distinctive developmental patterns when cultivated on 2% malt agar, resulting in either an appressed, yellow-brown pigmented colony or a white colony with extensive aerial mycelium. The switch from one colony type to the other sometimes occurred spontaneously, resulting in formation of sectors. On other occasions

it followed subculture, damage or inter- or intra-specific interactions with other mycelia. Juxtaposition of the two colony types resulted in formation of a dark brown pseudosclerotial plate. These observations suggest that endogenous mechanisms regulate the formation of different colony types.

During its potentially indeterminate life span, a fungal mycelium must be equipped to respond to a wide range of endogenously and exogenously induced changes in micro-environmental conditions. Thus it is well known that the thallus, as it expands from a germinating propagule, rapidly becomes structurally heterogeneous (Trinci, 1978). Whilst some of these structural changes can be related to direct environmental effects on wall growth and metabolism, others involve true differentiation of distinctive components of the thallus which can be said to exhibit different functional modes (Gregory, 1984). At the molecular level, the existence of such modes implies the involvement of regulatory mechanisms allowing selection of, and commitment to, developmental pathways which are distinctive due to differential gene expression.

Fungal differentiation has mostly been studied and discussed within the context of reproductive development, mycelial-yeast dimorphism and, to a lesser extent, the production of vegetative aggregates such as sclerotia and rhizomorphs (Smith, 1983). This has neglected many examples of vegetative pleomorphism, dualism and sectoring phenomena in mycelia. A striking example is found in certain wood-decaying Basidiomycotina in the genera *Hymenochaete*, *Phellinus* and *Inonotus* where the occurrence of two distinctive mycelial types, respectively characterized by appressed and aerial growth patterns, has been used as a diagnostic criterion in cultural identification (Stalpers, 1978). Here we describe a preliminary experimental investigation of this mycelial dimorphism in *Hymenochaete corrugata* (Fr.: Fr.) Lèv., and report how interaction between the two developmental states results in a pseudosclerotial plate. Pseudosclerotial plates (PSPs) are sheets of closely interwoven hyphae which are typically pigmented and pseudoparenchymatous (Campbell, 1934; Lopez-Real, 1975). When formed in natural substrata they delimit mycelial bodies, pseudosclerotia, and appear as lines ('zone lines') in cross-section, often adjacent to an exposed substratum surface or to an antagonistic neighbouring mycelium. Lopez-Real & Swift (1977) concluded that PSP formation is induced by damage to the mycelium, but no previous evidence of endogenous mechanisms affecting their development has been presented.

Isolates of *H. corrugata* from decayed wood were obtained from three different poles ('poles 1, 2 and 3') of hazel (*Corylus avellana* L.) originating from separate coppice stools in Friary Woods, a deciduous woodland near Bath (N.G. Ref. ST 783592). The samples were also colonized by *Hypoxylon fuscum* Pers.: Fr. and isolates of both fungi were prepared by plating out, onto 2% malt agar plus 0·01% novobiocin, surface-sterilized wood fragments, collected from cross-sections taken at intervals along the length of the poles. The cross-sections were also incubated separately in sealed polythene bags at ambient temperatures.

Isolates of *H. corrugata* from pole 1 exhibited the most clear-cut dimorphism, perhaps correlated with their slower extension rate than isolates from poles 2 and 3 (see below). In most cases the colonies developed either as an appressed mycelium ('flat' morphology type) which was pale luteous at first and acquired sienna and umber tints as it aged, or they developed as a purely floccose white growth with much aerial mycelium ('fluffy' morphology type) becoming pellicular with time (Fig. 1; nomenclature according to Rayner (1970) and Stalpers (1978)). Subcultures from colonies of one type commonly developed in the alternative form, and in some cases spontaneous reversion from one type to the other occurred (Fig. 1). All the isolates were somatically compatible, i.e. they intermingled without a rejection response when paired, cf. below, indicating that a single genotype was present throughout the 2·5 m length of the pole from which *H. corrugata* was isolated. However, when colonies of alternate form were juxtaposed, a chestnut/black PSP developed in all cases. A similar situation occurred in all cases where the different colony types developed on incubated wood sections and there was some indication that the flat type was associated with more decayed regions.

Isolates of *H. corrugata* from poles 2 and 3 were collected subsequently in order to confirm or extend the above observations, and showed some differences from those in pole 1. Whether these differences were intrinsic or due to different cultural procedures (pole 1 isolates were maintained in light-dark regimes at room temperature; pole 2 and 3 isolates were maintained at 20 °C in darkness) cannot be ascertained at this stage but, as will be explained, they probably have considerable significance in relation to regulation of the dimorphism. The most important departure from the behaviour previously observed was that all isolates from poles 2 and 3 appeared morphologically similar to one

Table 1. *Interactions between single basidiospore isolates from a fruit body of* Hymenochaete corrugata

Compatibility group 1				Compatibility group 2											
1	11	12	15	2	3	4	5	6	7	8	9	10	13	14	
I	P	W	P	C	C	C	C	C	C	C	C	C	C	C	1
	I	P	P	C	C	C	C	C	C	C	C	C	C	C	11
		I	P	C	C	C	C	C	C	C	C	C	C	C	12
			I	C	C	C	C	C	C	C	C	C	C	C	15
				I	W	W	P	P	P	P	W	P	W	W	2
					I	P	P	P	P	W	P	P	W	P	3
						I	W	P	P	P	W	P	P	P	4
							I	P	W	W	P	W	P	P	5
								I	W	P	P	W	P	W	6
									I	W	P	P	P	P	7
										I	P	W	P	P	8
											I	P	P	P	9
												I	P	P	10
													I	W	13
														I	14

Group 1: 1, 11, 12, 15. Group 2: 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 14.

C, compatible mating interaction and secondary mycelium formation; I, intermingling (somatically compatible interactions); P, pigmented zone (luteus to umber) developing between isolates; W, weak interactions.

another initially, having an effuse white floccose morphology and a considerably faster extension rate than those from pole 1. Dimorphism occurred later in development by formation of a sector or following subculturing, and some isolates showed no sudden transition to an alternative pattern of growth, only becoming progressively more pigmented as they aged from 3 to 4 weeks incubation onwards. As before, PSPs formed when alternative growth forms were juxtaposed, and all isolates from the same pole were somatically compatible, single genotypes occupying at least 1·65 and 0·80 m lengths respectively.

By contrast with isolates from the same pole, isolates from different poles were somatically incompatible when paired opposite one another on malt agar plates, a luteous-pigmented rejection zone containing hyphal ghosts, knots and spindle cells (see Rayner & Todd, 1979) developing between the colonies (Fig. 2). Hence, different poles contained different genotypes. In addition to the presence of somatic incompatibility, several other important features were noted in these pairings. In particular, unilateral or bilateral inhibition of colony extension and production of an appressed mycelial zone occurred in response to interaction. For example, unilateral inhibition of the pole 3 isolate by the pole 1 isolate resulted in the very clear demarcation of the former into the flat and fluffy colony types, separated by a PSP.

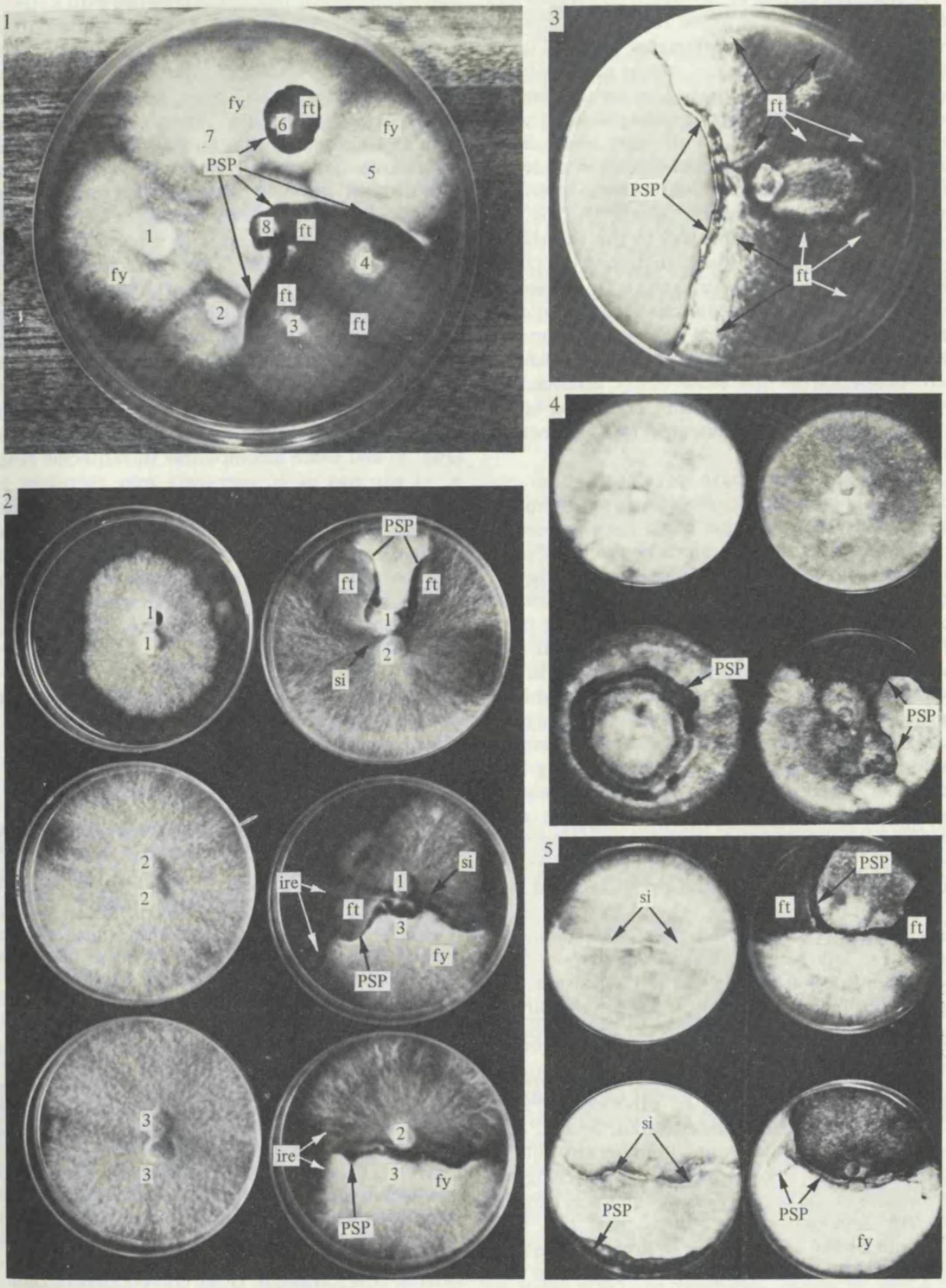
Fig. 1. Isolates of *Hymenochaete corrugata* from hazel pole 1, multiple inoculated onto a 2% malt agar plate. Isolates 1, 2, 5 and 7 have produced the 'fluffy' (fy) colony morphology. Isolates 3 and 4 have produced the 'flat' (ft) morphology. Isolates 6 and 8 have reverted from flat to fluffy morphology. Pseudosclerotial plates (PSP) have been produced at the interfaces between flat and fluffy colony types.

Fig. 2. Interactions between strains of *H. corrugata* isolated from hazel poles 1, 2 and 3. Control pairings are shown in the left-hand column: the more slowly extending, denser branching colony form from pole 1 compared with the faster extending, more effuse forms from poles 2 and 3 is evident. Interactions between different strains are shown in the right-hand column and exhibit somatic incompatibility (si), production of flat (ft) and fluffy (fy) sectors, pseudosclerotial plate production (PSP) and unilateral or bilateral inhibition of radial extension (ire) compared with controls.

Fig. 3. Interaction between a *H. corrugata* strain (left) and a *Hypoxylon fuscum* strain showing reversion to flat (ft) morphology and pseudosclerotial plate (PSP) formation associated with the contact region.

Fig. 4. Compatible matings between monobasidiospore isolates from a single fruit body of *H. corrugata*. PSP, pseudosclerotial plates.

Fig. 5. Incompatible matings. ft, flat morphology; fy fluffy morphology: si, somatic incompatibility reaction; PSP, pseudosclerotial plates.


1
fy
ft
6
7
fy
PSP
5
8
ft
1
4
fy
ft
2
3
ft
2
PSP
ft
ft
1
1
2
si
2
2
ire
si
1
ft
3
fy
PSP
3
3
2
ire
3
fy
PSP
3
ft
PSP
ft
4
PSP
PSP
5
si
PSP
ft
ft
si
PSP
fy

As indicated earlier, *H. corrugata* and *Hypoxylon fuscum* were commonly found together within the wood samples, and so experiments were set up to test the interactions between strains of these fungi in culture on 2% malt agar. The results showed some parallels with the intraspecific pairings in that the interactions resulted, in all cases where fluffy strains of *H. corrugata* were used, in a switch to flat morphology adjacent to the interaction interface, and concomitant production of a PSP (Fig. 3). Thereafter, the interaction was of the 'deadlock' type, with neither fungus able to make a territorial gain (cf. Rayner & Webber, 1984). In the wood, mutually exclusive decay columns occupied by each species and demarcated by a PSP were commonly observed; however, on some occasions both fungi were isolated from the same decay column, the non-demarcation perhaps being related to latent invasion colonization strategies (see Rayner & Boddy, 1985).

Hymenochaete spp. have previously been reported to be homothallic (Boidin, 1971; Boidin & Lanquetin, 1984), and this could have important implications for the interactions between different genotypes and the ability of homokaryotic lines to exhibit developmental dimorphism. We therefore obtained a set of single basidiospore isolates from a fruit body of *H. corrugata* collected from a fourth coppice pole. The isolates were paired in all combinations and the results are given in Table 1. As shown, although clamp connexions are absent in *H. corrugata*, evidence was found of a unifactorial homogenic incompatibility system governing formation of a heterokaryotic secondary mycelium, the isolates falling exactly into two groups with members of different groups being mating-type compatible (see also Fig. 4). Incompatible pairings, between members of the same group, resulted in a variety of interactions (Fig. 5) and in some cases there was evidence for switching of morphology in response to interaction. All the isolates resembled those from wood in poles 2 and 3. The existence of somatic incompatibility, and hence genetic difference, between strains from different coppice poles is therefore to be expected.

In conclusion, the occurrence of two colony types and their interaction to produce PSPs in *H. corrugata* has been demonstrated. Since PSPs probably have a protective and/or defensive role in nature, this has implications for the ecological significance of the capacity of the fungus to exhibit different developmental patterns, and this was borne out by the observations of growth form and PSP production on wood. Superimposed on the flat-fluffy dimorphism and somehow connected with it was a second type of dimorphism, between effuse colonies with a relatively rapid extension rate and more densely branching colonies with a slower extension rate; the flat-fluffy dimorphism was more distinct in the slower extending colonies and conversion to restricted radial extension was associated with interactions between different genotypes. Dimorphism between effuse colonies with fast extension rates and dense colonies with slow extension rates (slow, dense-fast, effuse dimorphism) has been observed with several fungi, and in certain strains of the basidiomycete *Stereum hirsutum* has been linked with a regulatory factor affecting a wide range of morphological features and somato-sexual responses of mycelia (Coates & Rayner, 1985*a*, *b*). The same factor has been linked with development of the 'bow-tie' interaction in *S. hirsutum*, which results in formation of an appressed mycelial zone widest at its edges between paired isolates (Coates & Rayner, 1985*c*). The occurrence of a similar zone in both interspecific (Fig. 3) and some incompatible intraspecific (Figs 2, 5) pairings of *H. corrugata* may therefore be significant. Systems such as that presently described for *H. corrugata* are clearly deserving of further experimental work, and may help to reveal mechanisms of functional compartmentalization which are fundamental to understanding of the physiology and ecological capabilities of mycelia of higher fungi.

Financial support to P.R.S. from the Bath University Research Fund is gratefully acknowledged.

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CYANIDE-RESISTANT RESPIRATION IN *CENOCOCCUM GEOPHILUM*

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The effects of cyanide, salicylhydroxamic acid and 2,4-dinitrophenol on oxygen uptake by intact mycelium of the ectomycorrhizal fungus *Cenococcum geophilum* support previous findings obtained with field-collected ectomycorrhizas by demonstrating cyanide-insensitive hydroxamic acid-sensitive alternative oxidase activity.

Cyanide-resistant 'alternative oxidases' which are inhibited by hydroxamic acid derivatives have been reported to occur in a number of plants and eukaryotic micro-organisms (Lambers, 1980). Several recent reviews (Solmos, 1977; Lambers, 1982; Laties, 1982) have addressed the occurrence and possible physiological significance of cyanide resistant respiration.

Harley and coworkers (Harley *et al.*, 1956; Harley & ap Rees, 1959) demonstrated the presence of a respiratory pathway resistant to cyanide and other inhibitors in beech (*Fagus sylvatica*) ectomycorrhizas, and Harley & ap Rees (1959) suggested that cyanide-resistant respiration was an attribute of the fungal sheath in these forms. Subsequently, Coleman & Harley (1976) demonstrated the presence of cyanide-insensitive alternate oxidase activity in mitochondria isolated from beech mycorrhizas; this activity was inhibited by the hydroxamic acid derivative mCLAM (m-chlorobenzyhydroxamic acid). Ectomycorrhizas of *Salix rotundifolia* and vesicular–arbuscular mycorrhizas (VAM) of *Salix nigra* also exhibit cyanide-resistant respiration, which can be inhibited to a great extent by SHAM (salicylhydroxamic acid) (Antibus, Trappe & Linkins, 1980).

At present information is lacking concerning the existence of cyanide-resistant respiration in ectomycorrhizal fungi grown in the absence of a host plant. The objectives of the present study were to determine whether cyanide-resistant respiration is present in ectomycorrhizal fungi grown in pure culture, to determine whether these fungi respond differently than field-collected mycorrhizas and to determine the extent of cyanide-insensitive respiration in vivo.

Isolates of *C. geophilum* from Barrow (VT 1004) and Cape Simpson, Alaska (VT 1005) were grown in liquid Hagem's medium as described by Antibus (1980). After 35 days of growth at 20 °C mycelial mats were harvested, washed and resuspended in 0·1 M-Na_2HPO_4 to NaH_2PO_4 buffer at pH 5·8. Oxygen uptake was measured using an Orbisphere oxygen electrode (Orbisphere Laboratories, York, Maine) calibrated with air-saturated water. The

following reagents were added in the sequences indicated for each experiment to give final concentrations of: KCN (5·0 mM), SHAM (2·5 mM) and 2,4-dinitrophenol (DNP) (0·5 mM). KCN solutions were neutralized with 0·1 N-HCl in a glass-stoppered volumetric flask over ice and used within 12 h of preparation. SHAM and DNP were prepared in 70% ethanol solutions. Mycelial oxygen uptake appeared to be unaffected by ethanol used at volumes similar to those employed in inhibitor studies. Each sequence of reagent addition was replicated three times. At the end of each run mycelial mats were washed, dried at 60° for 48 h and weighed.

Preliminary experiments with an isolate of *C. geophilum* from Maryland (VT 715) indicated that maximal inhibition of oxygen uptake was obtained with 1·0 mM-KCN. Additions of 5·0 mM-KCN reduced oxygen uptake in the Barrow and Cape Simpson *C. geophilum* isolates by 60–75% (Fig. 1). A similar concentration of KCN causes a 50% reduction in oxygen uptake by *S. rotundifolia* mycorrhizas (Antibus, Trappe & Linkins, 1980), while 4·2 mM-KCN reduces the oxygen uptake of beech mycorrhizas by 21–31% (Harley, McCready & Wedding, 1977). Therefore oxygen uptake by *C. geophilum* cultures appeared to be typified by a cyanide-insensitive component as found in ectomycorrhizal roots.

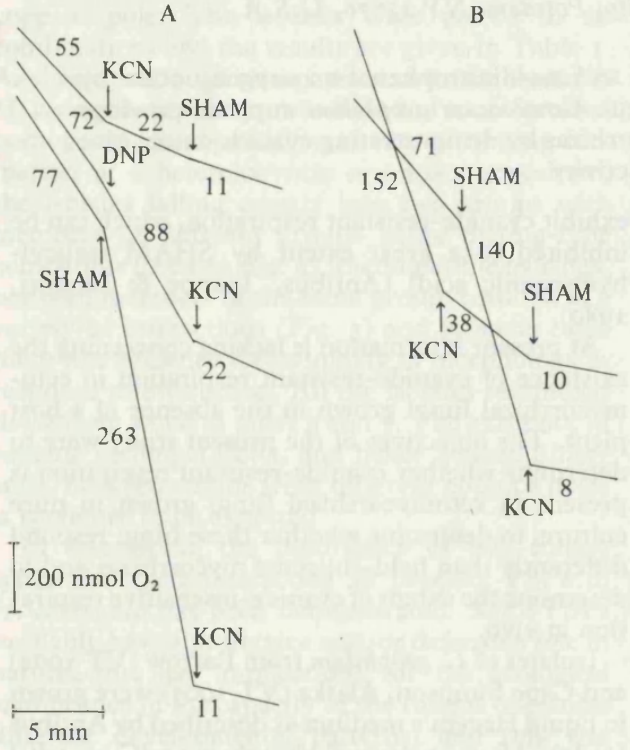

Fig. 1. Effects of various reagents on oxygen uptake by isolates of *C. geophilum* from (A) Barrow and (B) Cape Simpson, Alaska. Reagents were added to give the following final concentrations: KCN (5·0 mM), SHAM (2·5 mM) and DNP (0·5 mM). Numbers near lines are QO_2 values.

In preliminary experiments SHAM was added to KCN-inhibited cells of the Maryland isolate at final concentrations of 0·1, 0·5, 1·0, 2·0 and 3·0 mM. Inhibition of oxygen uptake occurred at the lowest concentration tested, with maximal inhibition obtained at 0·5–1·0 mM-SHAM. When 2·5 mM-SHAM was added to KCN-inhibited cells of the Barrow and Cape Simpson isolates the residual cyanide-resistant oxygen uptake was reduced by 50–75%, resulting in oxygen uptake values which were 7–20% of control values (Fig. 1). These data demonstrate the presence of cyanide-insensitive SHAM-sensitive alternative oxidase activity in *C. geophilum* cultures. Results obtained with willow and beech mycorrhizas indicate the presence of residual oxygen uptake which is not inhibited by a combination of KCN and hydroxamic acid derivatives. Likewise, *C. geophilum* cultures demonstrated residual oxygen consumption in the presence of KCN and SHAM.

Whereas it is possible to demonstrate the presence of a cyanide-resistant respiratory pathway in ectomycorrhizas and VAM, little information is available concerning the relative contribution of this pathway to total tissue respiration. Lambers (1982) suggested that the in vivo activity of this pathway is insignificant in these systems. A number of procedures have been employed to estimate the contribution of the alternative pathway to tissue respiration. One of these techniques involves examining the inhibitory effect of hydroxamic acids on oxygen uptake in the absence of KCN.

When SHAM was added to uninhibited mycelium of *C. geophilum* an immediate two to threefold stimulation of oxygen uptake was observed (Fig. 1). SHAM-stimulated oxygen uptake was significantly reduced by KCN addition, and residual oxygen uptake was similar to that obtained by adding KCN followed by SHAM. A similar stimulation of respiration by SHAM is observed in *S. rotundifolia* ectomycorrhizas, but not in *S. nigra* VAM (Antibus *et al.*, 1980). The observed difference in response to SHAM was attributed to the presence of a fungal mantle in the former and its absence in VAM. Our current findings show that SHAM stimulated the respiration of an ectomycorrhizal fungus in vivo.

Harley, McCready & Wedding (1977) observed stimulation of oxygen uptake, which they termed an 'uncoupling effect', in aged beech mycorrhizas. It was suggested that mCLAM was converted to an uncoupling agent in tissue, as it did not elicit such a response in isolated mitochondria. In further